STUDIES ON SEED FATS AND THEIR COMPONENT FATTY ACIDS

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
CHEMISTRY
TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH

ABDUL RAUF

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
1983

Dr. S. M. Osman
Professor of Chemistry and
Principal Invesligator, PL-480 Project.

Section of Oils & Fats
Department of Chemistry
Aligarh Muslim University
ALIGARH-202001 (India)

Ref. No.....

DATE 4.7.1983

THESIS SECTION

This is to certify that the work embodied in this thesis entitled, 'Studies on Seed Fats and their Component Fatty Acids' is the original work of Mr. Abdul Rauf done under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

(S.M. Osman)

With Love
To Parents

Acknowledgements

I wish to express my deep gratitude to Prof. S.M. Osman for his guidance and encouragement through out this research work. Thanks are due to Prof. W. Rahman, Chairman, Department of Chemistry for providing necessary facilities, Prof. M.S. Ahmad and Dr. D.K. Bhattacharya for helpful discussions.

I am deeply indebted to Drs. Fasih Ahmad and M. Shamim Ahmad for their generous help and suggestions. In addition I thank my colleagues who have rendered assistance in various ways. I am thankful to the instrumentation services of AMU, Aligarh; RSIC, Lucknow and RRL, Jammu for recording the spectra.

Lastly I acknowledge the CSIR, New Delhi for financial assistance.

A. Rauf

(Abdul Rauf)

Contents

Summary

Two types of investigative results have been described in the thesis. The Part I embodies the fatty acid and glyceride compositional studies on indigenous seed oils. The Part II relates to the results of synthetic work on long-chain α,β-unsaturated and epoxy fatty compounds.

Part I

In a continuing programme of chemical screening of the wild oil-yielding species to explore the oil seed potential of forest flora, few oils have been examined for their gross fatty acid profile and composition of their glycerides.

A. Cyclopropenoid Fatty Acids in Seed Oil of *Sterculia colorata* (Sterculiaceae)

The seed oil of *S. colorata* which responded to Halphen test and showed characteristic spectral data for cyclopropenoid fatty acids has been analyzed by using GC-MS of silver nitrate-methanol treated derivatives. The gross fatty acid composition found by GLC analysis in conjunction with GC-MS data showed the following composition: 12:0, 1.7; 14:0, 2.6; 16:0, 41.5; 18:0, 0.5; 18:1, 11.0; 18:2, 20.3; 18:3, 7.9; malvalic, 6.8 and sterculic, 7.5%.

B. Palmitoleic Acid in Ochna artopurpuria (Ochnaceae) Seed Oil

The seed oil of O. artopurpuria was found to be very unusual as compared to the fatty acid profile of the other members of Ochnaceae. Palmitoleic (cis-9-hexadecenoic) acid was characterized as an unusual component of the oil as evidenced by GLC of its degradative products. The fatty acid composition was found to be: 16:0, 34.1; 16:1, 25.6; 18:0, 0.4; 18:1, 20.3 and 18:2, 19.6%.

This species is the first member of Ochnaceae that contains palmitoleic acid, not so far reported to be a constituent of the glycerides of Ochnaceae oils.

C. Studies on Glyceride Structure of Seed Oils

Of the three seed oils Ochna squarrosa, O. artopurpuria and Zanthoxylum alatum, the first species is a hitherto unknown palmitic-rich seed oil. On the other hand latter two species yield oils rich in palmitoleic acid. The occurrence of palmitic and palmitoleic acids in these oils as predominant acids necessitated a study of their component glycerides. The glyceride composition of each oil has been determined by a combination of methods involving TLC, GLC and lipolysis. Using 1,3-random-2-random distribution pattern the calculated composition of the component glycerides is shown in the table.

Table

Seed Oils	Glycerides, mol %			
	GS_3	GS_2U	GSU_2	GU_3
Q. squarrosa	36.83	44.14	16.91	2.12
Q. artopurpuria	0.18	26.59	48.51	24.58
Z. alatum	0.04	10.63	43.93	44.97

A comparison of the glyceride compositional data reveals that Q. squarrosa oil triglycerides are composed mainly of GS_3 and GS_2U. This was expected due to the high content of palmitic acid (70.3%). The two species Q. artopurpuria and Z. alatum contain traces of GS_3 but the predominant glycerides are GSU_2 and GU_3, respectively. The high content of GSU_2 and GU_3 in Z. alatum is consistent with its gross fatty acid profile.

Part II

In this part the results of a variety of reactions on long-chain α,β-unsaturated compounds carried out for the first time are described. The structures of reaction products have been established by the use of combustion and spectral data.

D. Allylic Oxidation of trans-2-Enoates

Allylic oxidation of methyl trans-2-hexe-(1) and octadecenoate (2) with chromium trioxide in acetic anhydride and acetic acid yielded methyl 4-oxo-trans-2-hexa-(3) and octadecenoate (4) (Scheme 1).

Scheme 1

$$R\text{-}CH_2\text{-}CH{=}CH\text{-}CO_2CH_3 \xrightarrow[Ac_2O\text{-}AcOH]{CrO_3} R\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH{=}CH\text{-}CO_2CH_3$$

(1,2) (3,4)

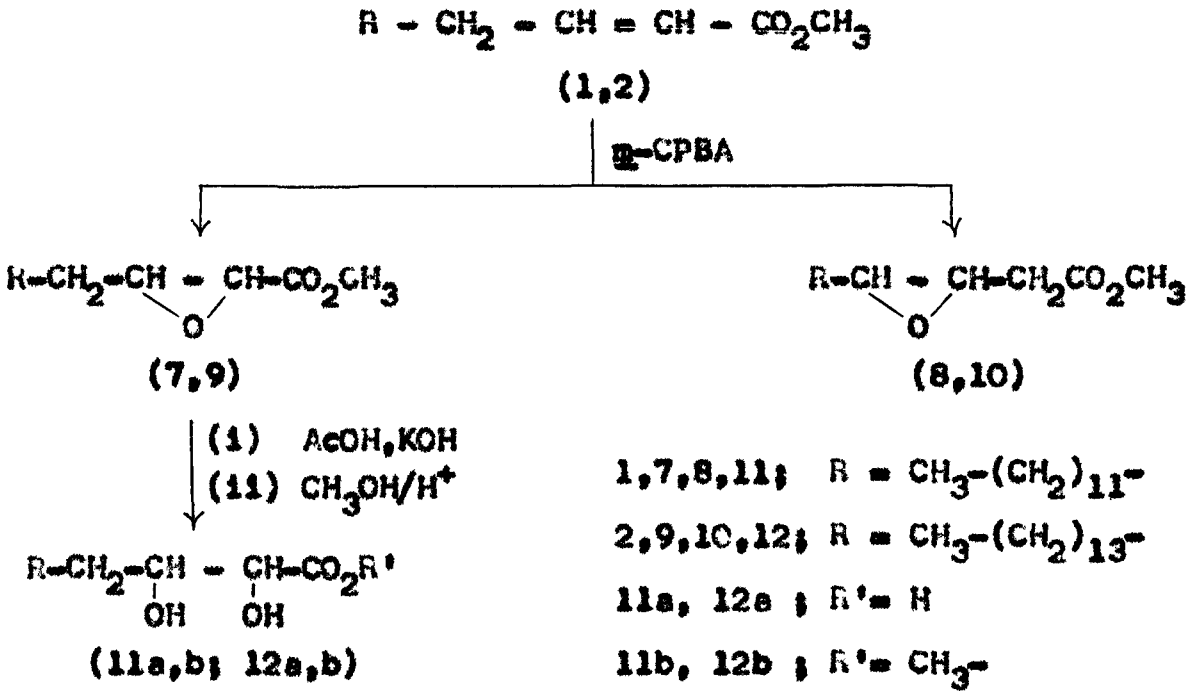
1,3; $R = CH_3\text{-}(CH_2)_{11}\text{-}$

2,4; $R = CH_3(CH_2)_{13}\text{-}$

E. Peracid Oxidation of α,β-Unsaturated Esters and their Derivatives

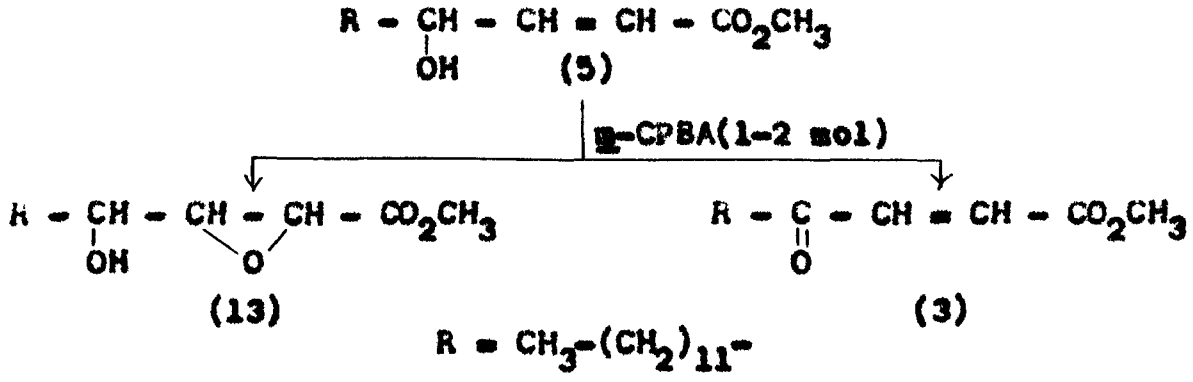
Peracid oxidations (epoxidation) of methyl trans-2-hexa-(1) and octadecenoate (2), methyl 4-hydroxy-trans-2-hexadecenoate (5), trans-2-octadecen-1-ol (6) and methyl 4-oxo-trans-2-hexa-(3) and octadecenoate (4) are reported. Reaction of 1 and 2 with m-chloroperbenzoic acid (m-CPBA) yielded trans-2,3-epoxy (7,9) as major and cis-3,4-epoxy (8,10) as minor products (Scheme 2).

Scheme 2



Compound 5 on oxidation with m-CPBA (1 mol) yielded methyl 4-hydroxy-trans-2,3-epoxyhexadecanoate (13). A similar reaction with 2 mol of m-CPBA afforded 13 along with a minor product 3 (Scheme 3).

Scheme 3



Epoxidation of 6 with m-CPBA furnished trans-2,3-epoxyoctadecan-1-ol (14) in quantitative yield (Scheme 4).

Scheme 4

(6) (14)

R = CH_3-$(CH_2)_{13}$-

Compounds 3 and 4 on m-CPBA oxidation gave rearranged products; 1-(methylcarboxy)-2-(dodecylcarboxy) ethylene (15) and 1-(methylcarboxy)-2-(tetradecylcarboxy) ethylene (16), respectively (Scheme 5).

Scheme 5

(3,4) (15,16)

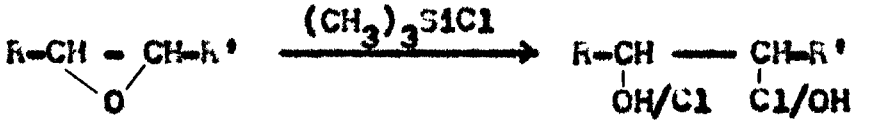
3,15; R = CH_3-$(CH_2)_{11}$-

4,16; R = $CH_3(CH_2)_{13}$-

F. Epoxy Ring Cleavage by Chlorotrimethylsilane[$(CH_3)_3$-SiCl]

A rapid and clean method for preparing chlorohydrins from long-chain epoxides by using $(CH_3)_3SiCl$ as a ring-opening reagent is described. Epoxy compounds (7,14,18,19) furnished the isomeric mixture of chlorohydrins (20a,b, 21, 23, 24) respectively while terminal epoxide (17) gave only one isomer viz: methyl 12-chloro-11-hydroxyundecanoate (22) (Scheme 6). Interestingly, the isomeric 2-chloro-3-hydroxy (20a) and 3-chloro-2-hydroxy (20b) derivatives of 7 were successfully separated by column chromatography.

Scheme 6



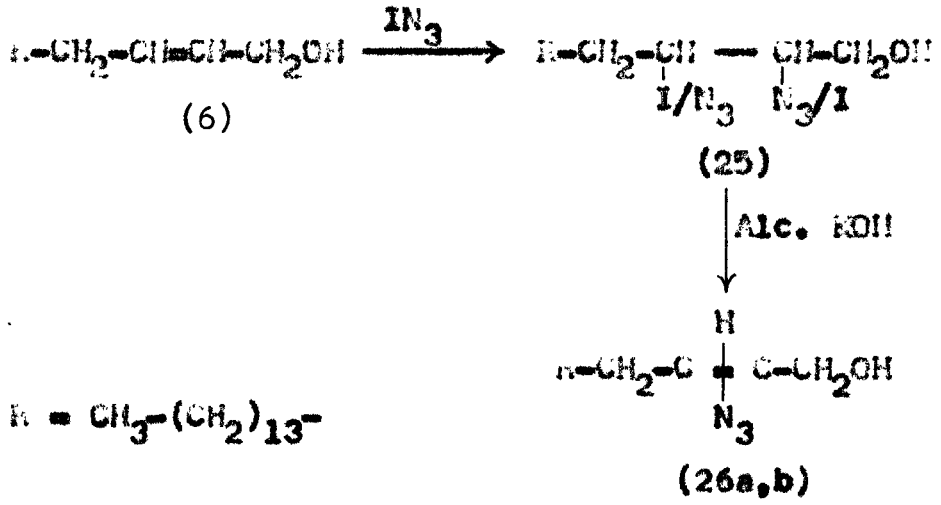
(7,14,17-19) (20a,b, 21-24)

Compound	R	R'
7,20a,b	$CH_3-(CH_2)_{12}-$	$-CO_2CH_3$
14,21	$CH_3-(CH_2)_{14}-$	$-CH_2OH$
17,22	H	$-(CH_2)_8-CO_2CH_3$
18,23	$CH_3-(CH_2)_7-$	$-(CH_2)_7-CO_2CH_3$
19,24	$CH_3-(CH_2)_4-$	$-CH_2-CH=CH-(CH_2)_7-CO_2CH_3$

G. Iodine-Azide (IN_3) Addition to trans-2-Octadecen-1-ol (6)

Addition of IN_3 to 6 yielded erythro-2(3)-azido-3(2)-iodooctadecan-1-ol (25). Treatment of 25 with a base afforded the isomeric vinyl azide (26a,b). The quantitation of 2-azido-(26a, 76%) and 3-azido-cis-2-octadecen-1-ol (26b, 24%) was based on NMR (Scheme 7).

Scheme 7

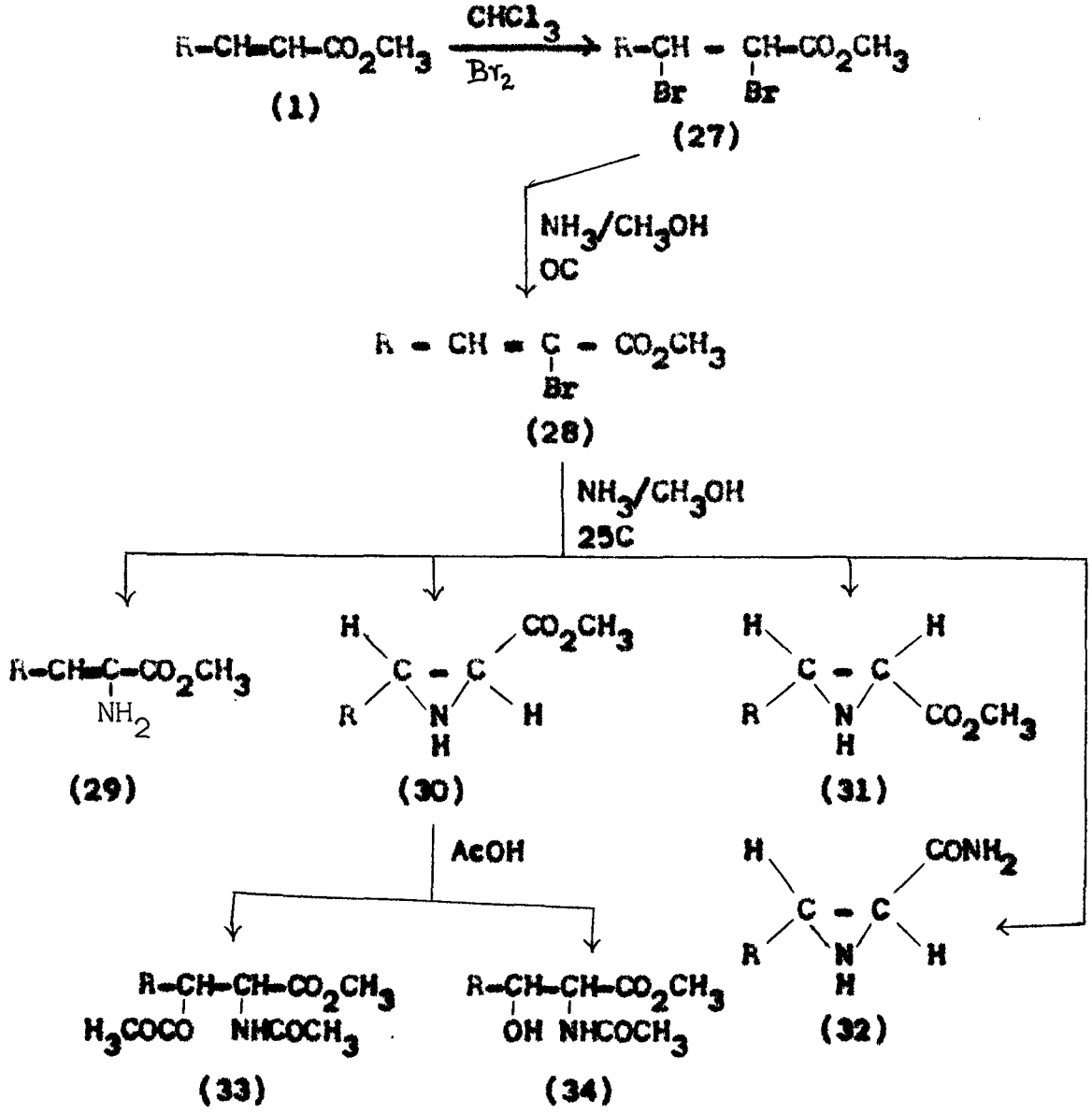


H. Aziridines from α,β-Unsaturated Ester

Reaction of methyl 2,3-dibromohexadecanoate (27) with ammonia at 0C gave methyl 2-bromo-2-hexadecenoate (28). Compound 28 on treatment with ammonia at 25C furnished methyl 2-amino-2-hexadecenoate (29), methyl trans-2,3-epiminohexadecanoate (30), methyl cis-2,3-epiminohexadecanoate (31) and trans-2,3-epiminohexadecamide (32). Ring fission of 30 with

acetic acid yielded 2-acetamido-3-acetoxy (33) (minor) and 2-acetamido-3-hydroxy derivative (34) (major). The products are depicted in scheme 8.

Scheme 8



R = $CH_3-(CH_2)_{12}-$

Introduction

Next to petroleum, the two major items of import for India are edible oils and fertilizers. It is the continuous shortage of edible oils that has contributed to escalating inflation and drainage of foreign exchange. During the last decade the production and utilization of edible fats and their fatty acids have grown both in size and diversity. In the industrial field there has been a competition between oleochemicals and petochemicals. Traditionally oleochemicals, while lacking an identity recognized by the general public, are essential to the production of a wide range of consumer goods. A variety of new useful products can be prepared by taking advantage of the inheritantly present functional groups in fatty acids, some of them could be substitutes for petroleum products. This approach has created an active interest in recent years in the study of derivatization of fatty acids. The recent hike in the cost of petrochemicals has diverted the attention of the technologists to find alternative sources of petrochemicals. Being renewable sources, vegetable oils can supplement the petrochemical replacement in addition to their essential edible uses. The efficient glyceride analysis techniques are gradually increasing the understanding of their structural composition. Thus the non-food uses of fats are solely dependent on the triglyceride composition which forms the basis of industrial utilization.

Fats and oils are found increasingly to be physiologically significant and chemically interesting. Renewed activity has arisen from the recognition of fatty acids as essential dietry requirement, from their link with the prostaglandins, and from their involvement in cell structural membranes. These discoveries followed principally from the development of improved chromatographic and spectroscopic techniques for studying these compounds.

India, primarily an agricultural country abounds in forest flora. There is a wide potential of agrichemicals derived from the minor oilseeds rich in specific kind of fatty acids. In recent years the utilization of fatty acids as agrichemicals find their way into a variety of industrial uses and most of them mainly through derivatization. One of the most exciting properties of fatty acid derivative is their insecticidal and antimicrobial activity.

In the context of chronic shortage of edible oils, the minor oilseeds found abundant in forest flora need chemical screening for exploring their industrial potential. Thus phytochemical screening of indigenous flora could provide additional vegetable oils which are probably the only economically feasible alternatives to petrochemicals as sources of long-chain aliphatic compounds. Keeping in view this objective an attempt has been made in the present study to carry out compositional studies on minor seed oils and to synthesize new compounds from the easily available fatty acid substrates.

Part I

Studies on Seed Oils And Glycerides

Theoretical

HBr-Reacting Fatty Acids

Among the naturally occurring fatty acids, conjugated dienol, epoxy and cyclopropene group-containing fatty acids react quantitatively with hydrogen bromide (HBr) under the conditions prescribed by the American Oil Chemists' Society (AOCS)[1]. Recently the latter two types of fatty acids have attracted attention of lipid chemists. The three HBr-reacting fatty acids are described below under the separate headings :

(a) Conjugated Dienol Acids

The fatty acids with a hydroxyl group alpha to conjugated diene grouping were shown to be present in the seed glycerides of a number of plants. The first of these to be characterized was dimorphecolic (9-hydroxy-trans-10, trans-12-octadecadienoic) acid present in Dimorphotheca aurantiaca (D. sinuata, Compositae)[2,3]. Morris et al.[4] and Chisholm and coworker[5] analyzed various species of Compositae and they characterized independently a mixture of two isomeric acids; 9-hydroxy-10,12- and 13-hydroxy-9,11-octadecadienoic acids. It was shown that these acids were either cis, trans or trans, cis in configuration. 13-Hydroxy-cis-9, trans-11-octadecadienoic (coriolic) acid was isolated and characterized by Tallent et al.[6] from Coriaria ruscifolia(Coriariaceae). Badami

and coworker[7] reported the presence of only 9-hydroxy-trans-10, cis-12-octadecadienoic acid in the seed oil of Calendula officinalis (Compositae). The cooccurrence of 9-hydroxy-trans-10,cis-12- and 13-hydroxy-cis-9,trans-11-octadecadienoic acids was reported by Powell et al.[8] in the seed oil of Xeranthemum annum (Compositae). The component acids of Monnina emarginata seed oil contained 13(S)-hydroxyoctadeca-cis-9,trans-11-dienoic acid (~ 30%), enantiomeric with the (R)-coriolic acid. Although these acids are mainly present as triglyceride, some of the acid (~ 4%) occurred as the lactone (a 14-membered ring)[9].

(b) Epoxy Acids

Fatty acids with epoxy groups occur naturally in seed oils of a considerable number of plant species. Krewson[10] reviewed the literature on epoxy seed oils. Earle[11] has supplemented Krewson's review and provided a comprehensive list of seeds in which epoxy oils have been found. The structure of vernolic (cis-12,13-epoxyoctadec-9-enoic) acid, isolated from Vernonia anthelmintica seed oil, was correctly elucidated by Gunstone[12]. Since then a fair number of 1,2-epoxy fatty acids have been isolated from the seed oils[13] including coronaric (cis-9,10-epoxyoctadec-12-enoic) acid[11] and 9,10-epoxy-trans-3, cis-12-octadecadienoic acid[14]. Recently the vernolic acid has

been found in seed oil of Hibiscus cannabinus[15] and Cephalaria syriaca[16]. As a variant of vernolic acid group, Crepis conyzaefolia (Compositae), reported by Spencer[17], contains vernolic acid and two previously unknown acids; (±) cis-12,13-epoxyoctadec-trans-6,cis-9-dienoic (14%) and cis-12,13-epoxyoctadec-cis-6,cis-9-dienoic (2%) acids.

Recently a C_{20} homologue of vernolic acid, named alchornic [(+)cis-14,15-epoxy-cis-11-eicosenoic] acid has been isolated from Alchornia cordifolia (Euphorbiaceae) seed oil by Kleiman et al.[18] Conacher and Gunstone[19] characterized a new epoxy acid, cis-9,10-epoxyoctadec-12-ynoic acid as a minor component of Helichrysum bracteatum (Compositae) seed oil, along with previously identified coronaric acid. Ulchenko et al.[20] analyzed the seed oil of Onopordium acanthium and reported the presence of mono(epoxy)octadecenoyl diacyltriglycerides which consisted of 63.1% α- and 36.9% β-epoxytriglycerides.

In the auther's laboratory, a new epoxy acid, cis-3,4-epoxy-cis-11-octadecenoic acid has been isolated from the seed oil of Vernonia roxburghii[21] along with vernolic acid. Mucuna prurita[22] (Leguminosae) was also found to contain a previously unidentified, cis-12,13-epoxy-trans-9-octadecenoic acid along with vernolic acid. Recently the seed oils of Mucuna pruriens[23], Hibiscus mutabilis[24], V. volkameriaefolia[25]

and *Abelmoschus moschatus*[26] have been found to contain vernolic acid in varying amounts.

The epoxy acids may be regarded as derivatives of oleic, linoleic and linolenic acids, in which one of the usually present double bond is epoxidised through metabolism. Seed oils rich in epoxy acids are of potential interest as replacement for synthetic epoxy compounds used as stabilizers for plastic materials[27] and also as starting material for the preparation of other derivatives. Swern *et al.*[28] have tested epoxy compounds for carcinogenic activity by repeated subcutaneous injection in mice.

(c) Cyclopropenoid Fatty Acids

Cyclopropenoid fatty acids (CPFA) occur most commonly in the seed oils of the order Malvales(Sterculiaceae, Malvaceae, Tiliaceae and Bombaceae)[29-32]. Recently Vickery[33] reported that CPFA occur randomly in small amounts in at least six other families; Anacardiaceae, Celestraceae, Sapindaceae, Ebenaceae, Sapotaceae and Rhamnaceae. Sterculic acid (SA, I) and malvalic acid (MA, II) often occur together and some times may be accompanied by small amounts of their dihydroderivatives.

Two other cyclopropenoid fatty acids have been discovered; D-2-hydroxysterculic (2-HSA,III) acid in the seed oil

$$CH_3 - (CH_2)_7 - C = C - (CH_2)_n - CH - COOH$$
$$\qquad\qquad\quad CH_2 \qquad\qquad\quad R$$

(I) n = 6; R = H, sterculic acid (SA)

(II) n = 5; R = H, malvalic acid (MA)

(III) n = 6; R = OH, 2-hydroxysterculic acid (2-HSA)

of Pachira insignis[34], P. aquatica[35] (Bombacaceae), Bombacopsis glabra[34] and sterculynic (8,9-methyleneoctadec-8-ene-17-ynoic) acid in seed oil of Sterculia alata[36] (Sterculiaceae). Recently Badami et al.[37] have reported only the occurrence of MA(17.6%) and SA(4%) from the S. alata seed oil.

Over a period of years, other investigators have found CPFA with chain length shorter than malvalic acid in various species. Raju and Reiser[38] reported evidence for CPFA shorter than MA in Althea rosea (Malvaceae) seed oil by GLC retention time. Johnson et al.[39] hinted at the occurrence of C_{17}-cycloprepene in the fruit of certain Malva species. Ackman and Hooper[40] encountered an unusual component in Euphoria longana (Sapindaceae) seed oil which they regarded as possibly being a C_{12}-cycloprepenoid acid.

The CPFA may occur with epoxy acids in many seed oils[13]. Recently Behannon et al.[35] reported the occurrence of CPFA along with epoxy acid (2.7%) from the seed oil of Pterygota alata (Sterculiaceae). They also reported the pre-

sence of hydroxy-conjugated diene acid from the seed oil of Hibiscus syriacus and Radyera farraqli along with CPFA.

More recently Berry[41,42] reported the presence of CPFA in Sterculia monosperma, Gnetum gnemon and Durio zibethinus seed oils. Abutilon pannosum seed oil was also found to contain CPFA[43]. (Ralaimanarivo et al.[44] reported the presence of CPFA from the six species of Adansonia.)

A number of workers shewed that the concentration of MA may usually be greater than those of SA in Malvaceae. Madrigal et al.[45] have reported that Pavonia sepium seed oil is unusual in being the first member of the family Malvaceae in which the content of SA was found to be greater (7%) than that of MA (4%). Recently similar observation (SA> MV) was observed by Ahmad et al.[23,46] in seed oils of Urena lobata and Sida rhombifolia of the family Malvaceae. In Bombaceae and Sterculiaceae either MA or SA may predominate in about equal frequency. The seed oil of Eriolaena hookeriana[47] and Pterospermum acerifolium[35] contains more malvalic (25.8 and 32.2%, respectively) than sterculic (6.0 and 3.8%, respectively) while Tarrietia utilis[35] contains more sterculic (20.2%) acid.

From the author's laboratory, a number of seed oils have been analyzed for CPFA. These are : Sida acuta[46], Hibiscus sabdariffa[48], H. caesius, S. grewioides[49], Kleinhovia

hemsita, Guazuma tomentosa[30], Althaea officinalis, Pentapetes phoenicea[31], Abutilon indicum[32], H. ficulneus[33], H. mutabilis[24] and Abelmoschus moschatus[26].

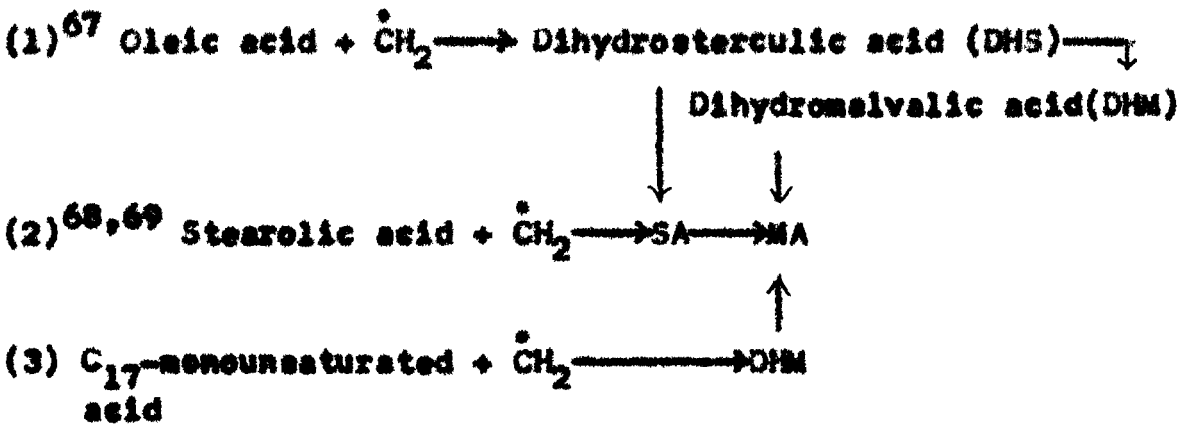
A small amount of dihydrosterculic acid (DHS) was reported in a large number of seed oils[33,35]. It also occurs as a major component (41%) in Litchi chinensis seed oil[34] and comprises 17% in the oil of Euphoria longana[35] (Sapindaceae). An investigation of seed oil of Byrsocarpus coccineus[36] (Connaraceae) disclosed the presence of lactobacillic (cis-11,12-methyleneoctadecanoic) acid in its oil. Lactobacillic acid has long been known as a constituent of certain bacterial lipids, but this is the first report of its presence in a seed oil.

The cyclopropene ring is the physiologically active entity of the two fatty acids (MA and SA). The physiological activity of SA is reported to be greater than that of MA[37]. It is well established that CPFA exert toxic and other adverse effects in a variety of animals[32,38]. Many physiological disorders in animals, including altered egg production and fertility in chickens, delayed sexual maturity and retarded growth in rats are attributed to cyclopropene ring[32,39]. CPFA were first reported to be cocarcinogenic[40,41] and then demonstrated to be carcinogenic in trout[42]. Recently Sinnhuber et al.[43] reported dietary CPFA to be a carcinogen. More recently it

was shown that these fatty acids decreased the levels of microsomal cytochrome P-450[64]. Selivonchicks et al.[65] reported that rainbow trout on treatment with CPFA altered the overall microsomal protein composition in a manner suggesting a reduction of high molecular weight components.

The Biosynthesis of CPFA in Plants

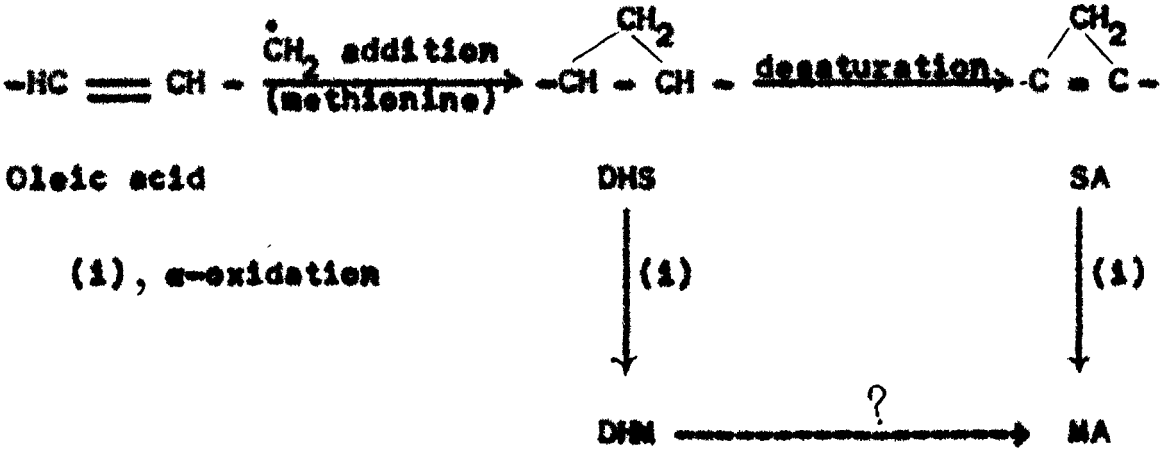
The mechanism of biosynthesis of cyclopropene fatty acids in higher plants are less understood and has been briefly reviewed[66]. Theories on the biosynthesis of the CPFA can be summarized as follows :



In the study of several ^{14}C-labelled compounds, Hooper et al.[70] and Johnson and coworkers[39] showed that L-methionine was the most efficient precursor of the ring methylene. From the variations in the labelling pattern with duration of incubations, they concluded that the probable

pathway was oleic ⟶ DHS ⟶ SA and that Bu'Lock's[68] suggestion of acetylenic precursors for the cyclopropane acid was not supported by their results. Evidence obtained by Yano *et al.*[71] suggests that in higher plants (Malvaceae) the CPFA biosynthetic pathway (Scheme 1) involves the initial formation of DHS from oleic acid, with subsequent desaturation to sterculic acid and α-oxidation to malvalic and DHM acids. They have confirmed that methionine, presumably as

Scheme 1



S-adenosyl methionine, is the methylene donor and that in the callus tissue cultures, in the medium used, methionine concentration may be rate limiting. There is no evidence that stearolic acid is involved in this sequence.

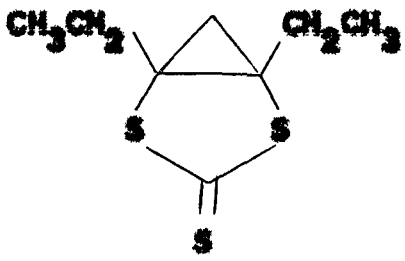
Quantitation and Characterization of CPFA

The quantitation of CPFA is of great importance because of their biological effects. The existing CPFA quantitation techniques have been found to be less than adequate and have resulted in a continuing search for a method with acceptable reliability and precision especially at the low range of levels found in products consumed in human diet. Essentially the following are methods used in the quantitation of CPFA :

(i) Halphen Test,
(ii) HBr-titration,
(iii) Gas-liquid Chromatography, and
(iv) Spectroscopic Methods.

(i) Halphen Test

It is well known that the CPFA containing seed oils responded to Halphen test[72], giving a red or orange colour. Zaheresky et al.[73] established the structure of coloured products (IV,V) formed by cyclopropene ring with Halphen reagent.



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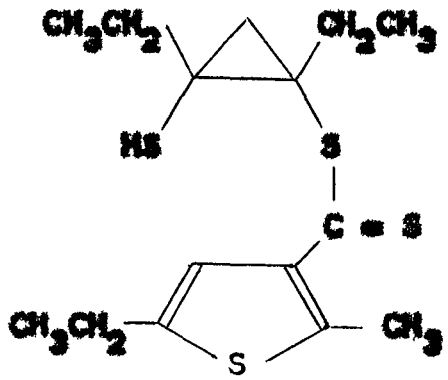
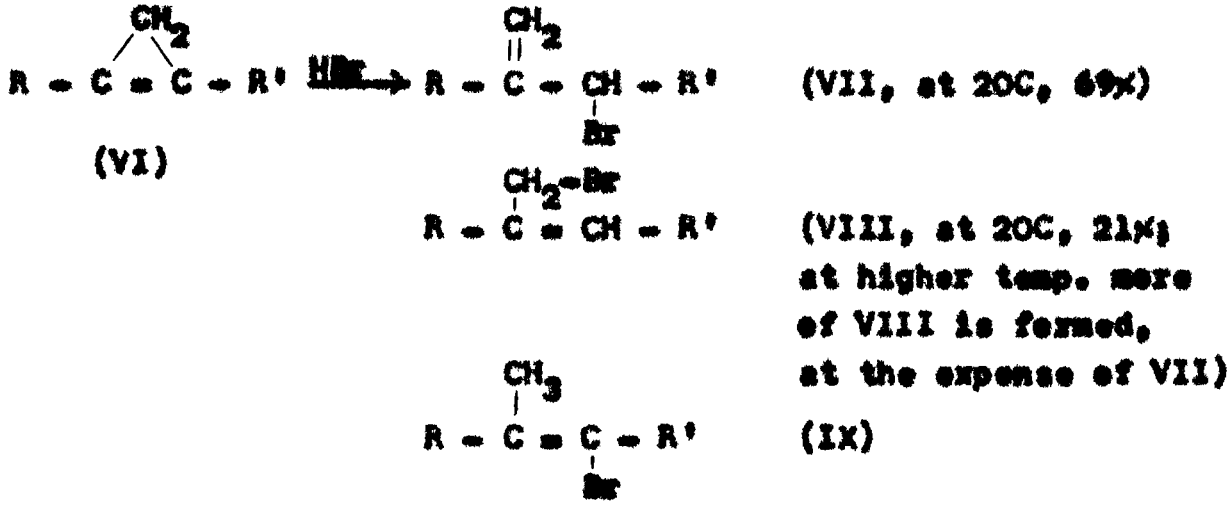


(V)

The Halphen reaction uses sulphur dissolved in carbon disulphide which reacts with cyclopropene ring to produce chromogen that is measured colorimetrically for quantitative analysis of CPFA[74-76].

(ii) <u>HBr-titration</u>

In this method the cyclopropene moiety (VI) reacts quantitatively with HBr to yield three types of compounds[77-80] (VII-IX).



Recently Feuge et al.[81] reported the improvements in the preparation of samples and the procedure of titration including the use of colour indicator, 4-phenylazodiphenylamine.

The crystal violet and the other colour indicators that had been used in HBr-titration, function poorly in toluene/benzene and the end point presented problems. A potentiometric titration that avoids these problems was devised recently by Zeringue et al.[82]

(iii) Gas Liquid Chromatography (GLC)

There are two basic methods used in the quantitation of CPFA by use of GLC : (a) Direct GLC of CPFA, and (b) GLC of CPFA Derivatives.

Reseurt et al.[30] used a direct GLC method for the analysis of methyl esters of several seed oils that contained 8-58% CPFA and have shown that the cyclopropene acids tend to isomerize and decompose as they pass through the GLC column. In addition GLC data show that the malvalic acid peak is masked with the linoleic acid peak[67]. A number of workers[38,83] have concluded that CPFA esters are too unstable for analysis by GLC methods. Recently Fisher et al.[84] described a method for the analysis of CPFA with a glass column packed with a methyl silicone substrate on a inner support and concluded

that methyl sterculate and methyl malvalate can be chromatographed without decomposition. Although methyl malvalate was not well resolved from methyl linoleate, it can be quantitated accurately at concentrations as low as 0.03% by a peak-height method. Quantitation can be done manually with an internal standard, or with a data system without an internal standard. The methyl malvalate and sterculate concentrations can be calculated from the concentration of nonadecanoate added as an internal standard or from the concentration found by peak-area normalization for heptadecanoate, stearate or hydrosterculate, by the equation :

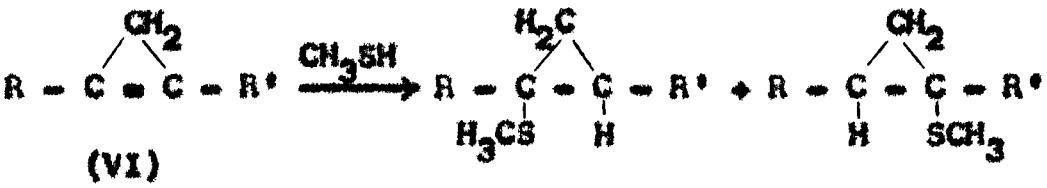
$$Cx = (CrHxTx)/HrTr$$

where; C, H and T are concentration, height, and retention time, x denotes malvalate or sterculate and r is one of the reference esters.

Selective derivatization of CPFA followed by GLC analysis of the products have been proposed as primary methods[38,83]. However, Coleman[85] found that method of Raju *et al.*[38] was unsatisfactory and that the method of Schneider *et al.*[83] was satisfactory at high CPFA level but not at the levels found in cottonseed oils. The chemical reactions involved in derivatization are : hydrogenation[67,86], reaction of mercaptans[87] (Scheme 2) and reaction with silver nitrate[83,88]

in methanol and non-hydroxy solvents (such as acetone, acetonitrile etc.).

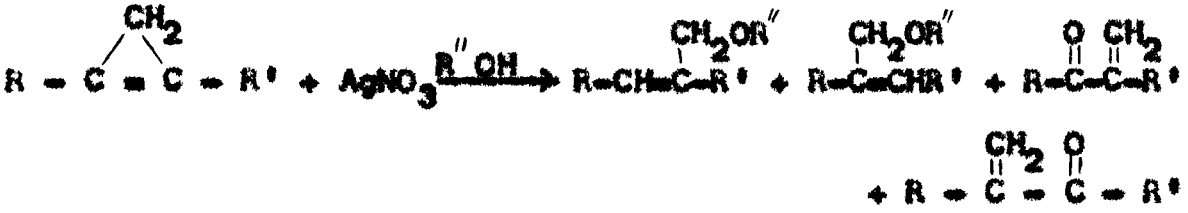
Scheme 2



(VI)

The following derivatives formed by treatment of $AgNO_3/R''OH$ (Scheme 3) and can be successfully used for GC-MS

Scheme 3



analysis[44,47]. From the auther's laberatory GC-MS of $AgNO_3$-treated methyl esters of E. heskeriana has been reported[47]. The utility of this reaction is that unsaturated components present initially remain unaffected and can be isolated and determined separately.

(iv) Spectroscopic Methods

Spectroscopic methods such as infrared (IR) and nuclear magnetic resonance (NMR) have been tried for quantitation of CPFA, but is of little practical importance. IR spectra of CPFA showed two distinct bands; one at 1008-1010 cm^{-1} which is attributed to the in-plane wagging vibration of the ring methylene group; the additional weaker band at 1852 cm^{-1} is ascribable to the stretching frequency of ring double bond. Measurement of the absorption at 1008-1010 cm^{-1} has been suggested as a means of estimating the total cyclopropene ring.

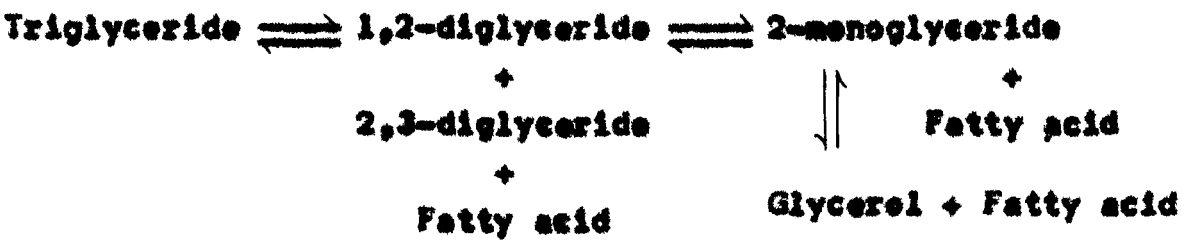
Pawlowski et al.[89] described the use of NMR as a rapid, simple and quantitative for cyclopropenoid function in lipids. Good accuracy is obtained at CPFA concentration of 1% to 100%. The position of absorption (τ 9.2) of two ring methylene protons is solvent dependent. They calculated percent CPFA by dividing the area from methyl absorption into the area from the cyclopropene absorption (and multiplying by 150).

Glyceride Composition by Lipolysis

The triglyceride composition of vegetable oils is of both academic and industrial interest. The triglyceride

composition of natural fats is so complex that no one analytical technique can define all the components present. The introduction of new analytical techniques for the investigation of glyceride structure has added great impetus towards a better understanding of the glyceride composition of natural fats. These methods include : (i) the rapid and widely applied silver-ion adsorption thin-layer chromatography for separating triglycerides into fractions differing only in unsaturation[90,91], (ii) GLC separation of triglycerides according to molecular weight[92], (iii) pancreatic lipase hydrolysis[90,93], (iv) liquid-liquid partition chromatography (LLC)[94], (v) glass capillary gas chromatography[95] and (vi) high pressure liquid chromatography (HPLC)[96].

The pancreatic lipase is widely used for studying the distribution of acyl groups in triglycerides based on its ability to hydrolyse the acyl groups attached to the primary glycerol hydroxyl groups and to leave intact the acyl group attached to the secondary hydroxyl group. Lipase catalyses the stepwise hydrolysis of triglyceride to glycerol via diglycerides and monoglycerides. The scheme is given as follows :



By careful examination it is possible to determine the component acids present in natural mixture of triglycerides and in the 2-monoglycerols derived from them by lipolysis. By simple calculation these provide the composition of fatty acids in the 2-position and in the combined 1- and 3-positions, but these data do not indicate directly about glyceride composition. Vander Wal[97] and Coleman and Fulton[98] independently suggested that it may be possible to calculate glyceride composition from lipolysis data if it is assumed that (i) the fatty acid attached to the 1- and 3- positions are the same and (ii) the fatty acids occupying the 1-, 2- and 3- positions are associated with each other in a random manner. Determination of the fatty acids at the glyceride 2-position, deduction of the composition of the acids of the 1- and 3-positions, and use of the 1,3-random-2-random distribution theory to calculate the triglyceride composition of a natural oil has led to a much greater understanding of oil compositions. The validity of the approach has been confirmed in numerous papers and publications[99].

The positional distribution of oleic, linoleic and linolenic acids in plant triglycerides has been recognized, but clearcut patterns have not been defined. Gunstone[100] and Mattson and Volpenhein[101] have suggested that 18:1, 18:2 and 18:3 are randomly distributed among the free hydroxyl group

remaining after 16:0, 18:0 and all > C_{18} acids are esterified at the α-positions. Further study of lipolysis results by Gunstone and coworkers[102] and Matteon and Volpenhein[101], however, indicated that among the unsaturated C_{18} acids, oleic and linolenic show a slight preference for the α-positions while linoleic shows a slight preference for the β-position. This was taken into account by Evans et al.[103] when they proposed a new positional distribution hypothesis following three rules : (i) saturated acids and those with chain lengths greater than 18 carbons are first distributed equally at the two α-positions, (ii) oleic and linolenic acids are then distributed equally and randomly on the unfilled α- and β-positions, with any excess from the α-positions being added to the β-position, (iii) all remaining positions are filled by linoleic acid.

Correlation studies on lipolysis data from twenty four species of Cruciferae seed triglycerides were carried out by Litchfield[104]. Studies on unfractionated triglycerides of Brassica species revealed that all saturated and long-chain (C_{20}-C_{24}) fatty acids are esterified at 1- and 3-positions, while unsaturated C_{18} acids are preferentially located at the 2-position[105]. Recently Zadernowski[91b] reported the order of preference for 2-position as linoleic> linolenic> oleic acids in medium and low erucic acid rapeseed oil.

Recently Pee *et al.*[106] studied positional distribution of fatty acids in triglycerides of *Mangifera indica* kernel fat. They showed that palmitic, stearic and arachidic acids were almost exclusively distributed among the 1- and 3-positions. Oleic acid represented 85-89% of fatty acids at 2-positions. Cole *et al.*[107] showed that *Jubaea spectabilis* (Cocoideae) oil maintains the tendency of all members of Cocoideae for unsaturated acids to appear at the 2-position, to the extent of 45 mole % and 55 mole % for the 18:1 and 18:2 acids respectively. Recently triglyceride characteristics of cocoa butter from cacao fruit was determined by Lehrian *et al.*[108]

Quedraogo and coworkers[109a] reported the triglyceride structure of sesame oil and showed that linoleic (43.3%) and oleic (40.0%) acids preferentially esterified in the 2-position. The oil of *Sesamum indicum*[109b] composed of 8, 41 and 5% S_2U, SU_2 and U_3 respectively.

The glyceride composition of the seed oil of *Thespesia populnea*[110] (Malvaceae) has been determined and resembles in many respects to that of Egyptian variety of cotton seed oil[111]. The triglyceride structure of a cotton seed oil from seed harvested in upper Volta (Africa) has been studied by Quedraogo and coworker[112]. The contents in linoleic, palmitic and oleic acids are 52.2, 24.5 and 19.5

respectively. The two unsaturated acids, especially, linoleic acid, are preferentially esterified in the 2-position of glycerol.

The triglyceride composition of Madhuca butyracea (Phulwara butter) was analyzed by Sengupta and coworker[113]. The special characteristic of Phulwara butter is its content of PPO, 52.5; PLP, 4.9; POSt, 8.6; POO, 14.4 and PP, 7.7% (P = Plamitic, St = stearic, O = oleic and L = Linoleic). 2-Monoglycerides obtained by lipolysis of this fat and its least soluble fraction contained 13.0 and 29.3% saturated acids. This butter may be a potential source of palmitic acid for industrial utility.

The seed oils of soybean[114], corn oil[115], Nicotiana rustica[116] and kernel of Simaruba glauca[117] have been studied for their glyceride structure.

The Unusual Glycerides

Glyceride studies have so far been made mainly on fats of industrial or medicinal interest. Our present knowledge about glycerides is fragmentary. Recent discoveries, include a variety of typical derivatives of glycerol which have been discovered in seed oils. Several seed oils containing hydroxy acids are known to have more than three fatty acids per glycerol molecule[9a,118]. The oil of Chamaesyce

afra[119] (Compositae) was found to be unusual in having glycerides containing more than three acyl group, that is, more polar penta-acid triglycerides. Plattner *et al.*[120] have reported unusual glycerides from *Heliophila amplexicaulis* (Cruciferae) and the hydroxy acids in this oil were found exclusively in the 1- and/or 3-position of triglycerides and are completely acylated with C_{20} or C_{22} saturated or monoenoic acids. The Kamala (*Mallotus philippinensis*)[121] seed oil has polyacid glycerides carrying from 3 to 8 fatty acids. Recently Madrigal *et al.*[122] showed the existence of tri-, tetra-, penta- and hexaacyl glycerols with no more than two fatty acid moieties on one acyl chain attached to one position of glycerol in *Irewia nudiflora*. Another series of glyceride derivatives have been identified as acetotriglycerides in the seed oil of *Polygala virgata*[123] and *Celastrus orbiculatus*[124] (Celastraceae). In the latter case the monoacetotriglycerides represent 68-98% of the seed oils from selected species in the Celastraceae. The glyceride structures of epoxy acid containing seed oils such as *Erlangea tomentosa*[125], *Wrightia tinctoria* and *Hentage benchalensis*[126] have also been studied.

Discussion

1.1. Cycloprepenoid Fatty Acids in Seed Oil of *Sterculia colorata*

Recently the CPFA have been the subject of much investigation due to their profound biological[127] effects on animals and cocarcinogenic properties[60,61]. As a part of screening programme aimed at the search of biologically active cyclopropene acids in minor seed oils, it was considered worthwhile to examine the seed oil of *S. colorata* (Sterculiaceae).

S. colorata, Roxb. (Sterculiaceae) vern. Bodula, samarri (Oudh) is a moderate-sized tree. It has crowded leaves at the ends of the branches. Seeds usually two, oval, smooth, pinkish outside. The tree is found throughout the forests of Rohilkhand, Bundelkhand and Oudh[128].

It was found that *S. colorata* seed oil responded to Halphen test[72], indicating the presence of CPFA. The IR spectrum of the crude oil showed a sharp band at 1010 and a weak band at 1032 cm^{-1}. The prominent NMR singlet at τ 9.28

Note: After completion of our work, Daulatabad and Ankalgi[142] reported the fatty acid composition of *S. colorata* seed oil. The presence of 8.1% CPFA in seed oil was detected using GLC of silver nitrate methanol-treated methyl ester, but no GC-MS studies of these methyl ester were carried out. The results are however different from our findings. The variations may be due to the differences in varietal and agronomic conditions.

for two methylene protons confirmed the presence of cyclopropene group. There was no UV maxima in the range 228-315 nm (conjugated unsaturation). Oil characteristics and seed properties were determined according to the procedures recommended by AOCS methods[1] and the data are summarized in table 1. HBr-titration[77] of oil at 55C, indicated HBr-reacting cyclopropene acid content of 13.6%.

Table 1

Analytical Data of S. colorata Seed Oil

Seeds	
Oil content (%)	27.7
Unsaponifiable content (%)	5.1
Protein content, Nx6.25 (%)	16.8
Moisture (%)	5.7
Seed Oil	
Iodine value (Wijs)	92.96
Saponification value	185.4
Refractive index, n_D^{30}	1.4770
Halphen test	Positive
HBr-equivalent	13.6

Analytical TLC revealed the presence of only non-oxygenated esters from S. colorata seed oil. The silver-ion

TLC showed distinct spots of saturates, monoene, diene and triene parallel to those obtained from S. foetida esters resolved alongside. An additional spot trailing just behind the spot of saturates was observed which was presumed to be due to the presence of CPFA in the seed oil. On a siliconised plate, the S. colorata esters showed clear spots of the usual critical pairs and a spot near the base line corresponded to cycloprepene esters, as did the S. foetida esters.

The fatty acid composition of the oil was determined by GC-MS study of silver nitrate-treated esters following the procedure of Schneider et al.[83] The GLC chromatogram clearly established the presence of malvalic and sterculic acids in S. colorata seed oil by a comparison of the relative retention times of the derivatives of S. foetida esters. The GLC data of this oil showed the presence of 14.3% by weight of CPFA. The amount of fatty acids obtained by GLC analysis was found close to that obtained by HBr-titration (Table 2).

The GC-MS analysis of silver nitrate-methanol treated methyl esters clearly demonstrated the presence of malvalic (6.8%) and sterculic (7.5%) acids, in addition to normal fatty acids. The MS of silver nitrate derivatives of methyl malvalate and methyl sterculate (Scheme 4, X-XVII) confirmed the GLC identification; in addition, spectra of

normal esters showed the appropriate molecular ions. Scheme 4 set out the expected products from each cyclopropene esters.

Table 2

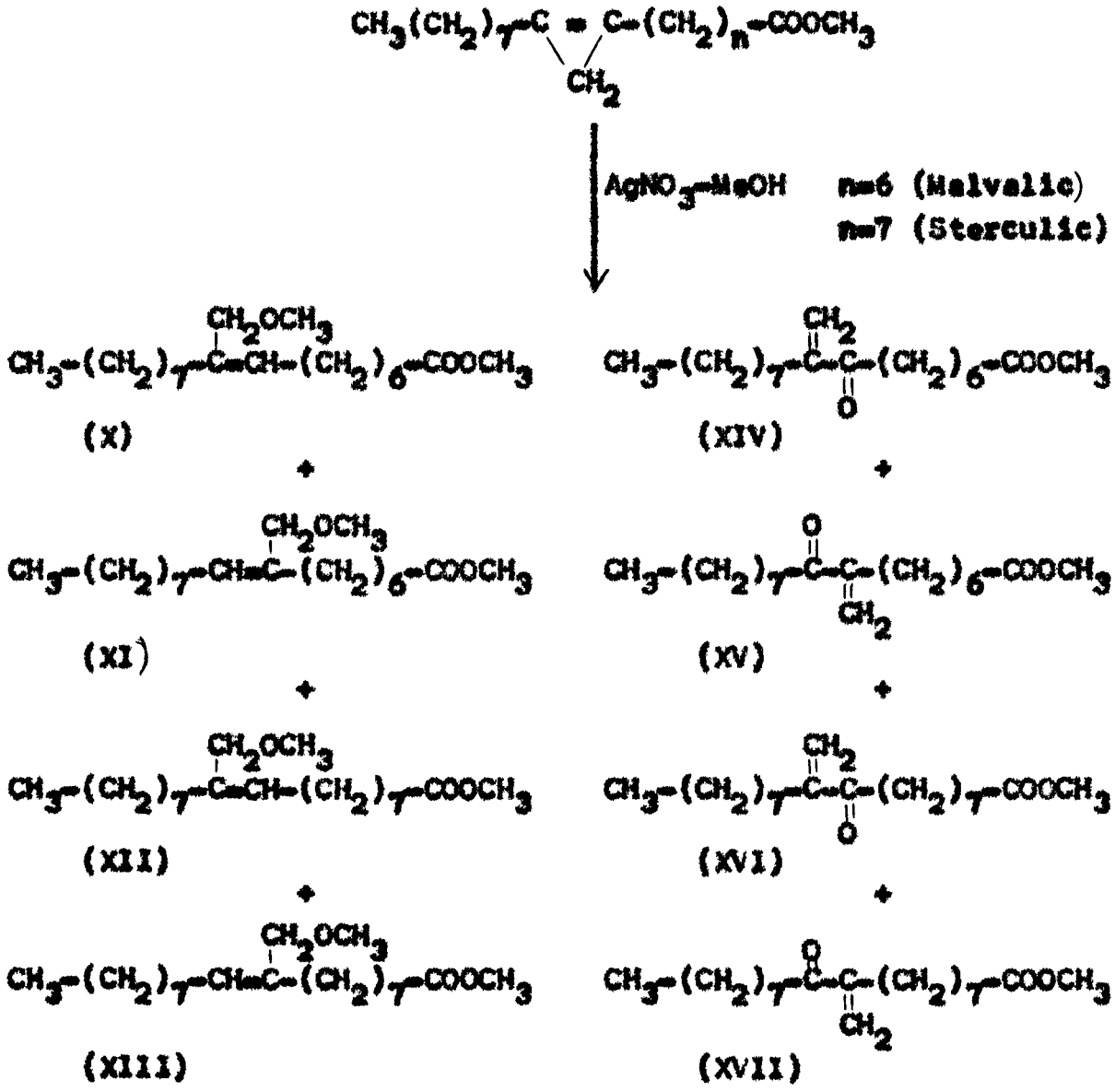
Component Methyl Esters (% Wt.) Derived from *S. colorata* and *S. foetida** Seed Oils.

Fatty acids	RRT**	S. foetida		S. colorata		M+
Lauric (12:0)	0.17	-		1.7		214
Myristic (14:0)	0.23	-		2.6		242
Palmitic (16:0)	0.47	26.0		41.5		270
Palmetoleic (16:1)	0.74	1.0		-		268
Stearic (18:0)	0.89	3.4		0.5		298
Oleic (18:1)	1.00	9.4		11.0		296
Linoleic (18:2)	1.20	1.3		20.3		294
Linolenic (18:3)	1.38	0.6		7.9		292
Malvalic (ether deriv.)	2.31	6.5	7.1	5.9	6.8	310
(Ketone deriv.)	4.20	0.6		0.9		
Sterculic (ether deriv.)	3.18	48.8	51.2	6.4	7.5	340
(Ketone deriv.)	5.74	2.4		1.1		324

*Reference standard of malvalic and sterculic acid.

**Relative retention time (RRT) is expressed by the ratio of retention time for the substance under examination to the retention time (10 min) for methyl oleate.

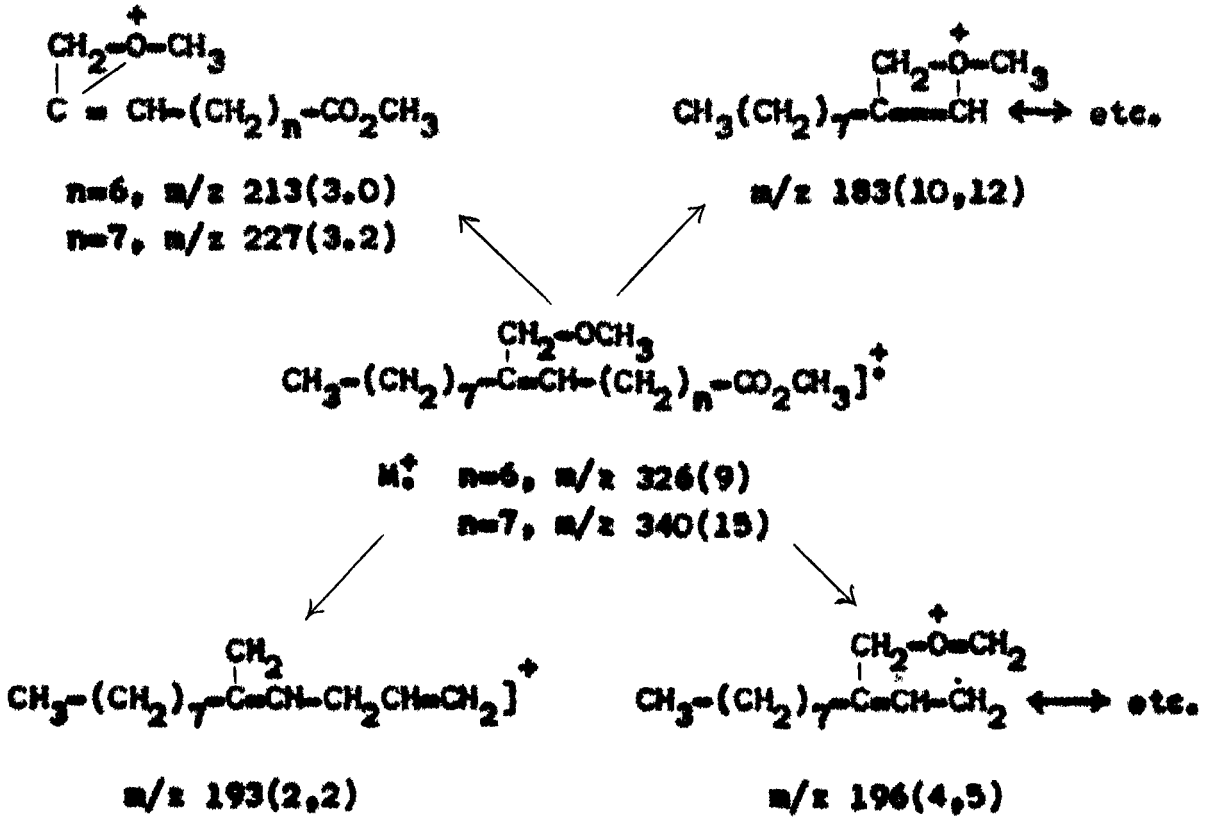
Scheme 4


CH3(CH2)7-C = C-(CH2)n-COOCH3
CH2
AgNO3-MeOH
n=6 (Malvalic)
n=7 (Sterculic)
CH2OCH3
CH3-(CH2)7-C=CH-(CH2)6-COOCH3
(X)
+
CH2OCH3
CH3-(CH2)7-CH=C-(CH2)6-COOCH3
(XI)
+
CH2OCH3
CH3-(CH2)7-C=CH-(CH2)7-COOCH3
(XII)
+
CH2OCH3
CH3-(CH2)7-CH=C-(CH2)7-COOCH3
(XIII)
CH2
CH3-(CH2)7-C-C-(CH2)6-COOCH3
O
(XIV)
+
O
CH3-(CH2)7-C-C-(CH2)6-COOCH3
CH2
(XV)
+
CH2
CH3-(CH2)7-C-C-(CH2)7-COOCH3
O
(XVI)
+
O
CH3-(CH2)7-C-C-(CH2)7-COOCH3
CH2
(XVII)

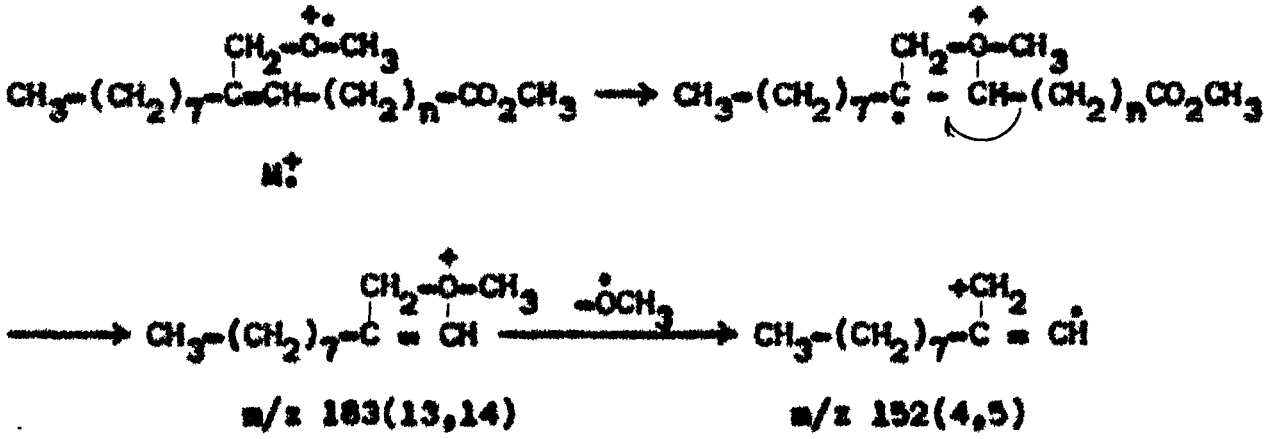
MS of Ether-Derivatives (X, XI and XII, XIII)

Methyl 9(8)-methoxymethylheptadec-8-enoate (X,XI) and methyl 10(9)-methoxymethyloctadec-9-enoate (XII,XIII) showed the molecular ion peaks at m/z 326 ($C_{20}H_{38}O_3$) and 340 ($C_{21}H_{40}O_3$). The diagnostic peaks which showed the position of cyclopropene ring in X and XII are given in scheme 5.

Scheme 5



A peak at m/z 152 is believed to arise from m/z 183 by the loss of m/z 31 as shown below :

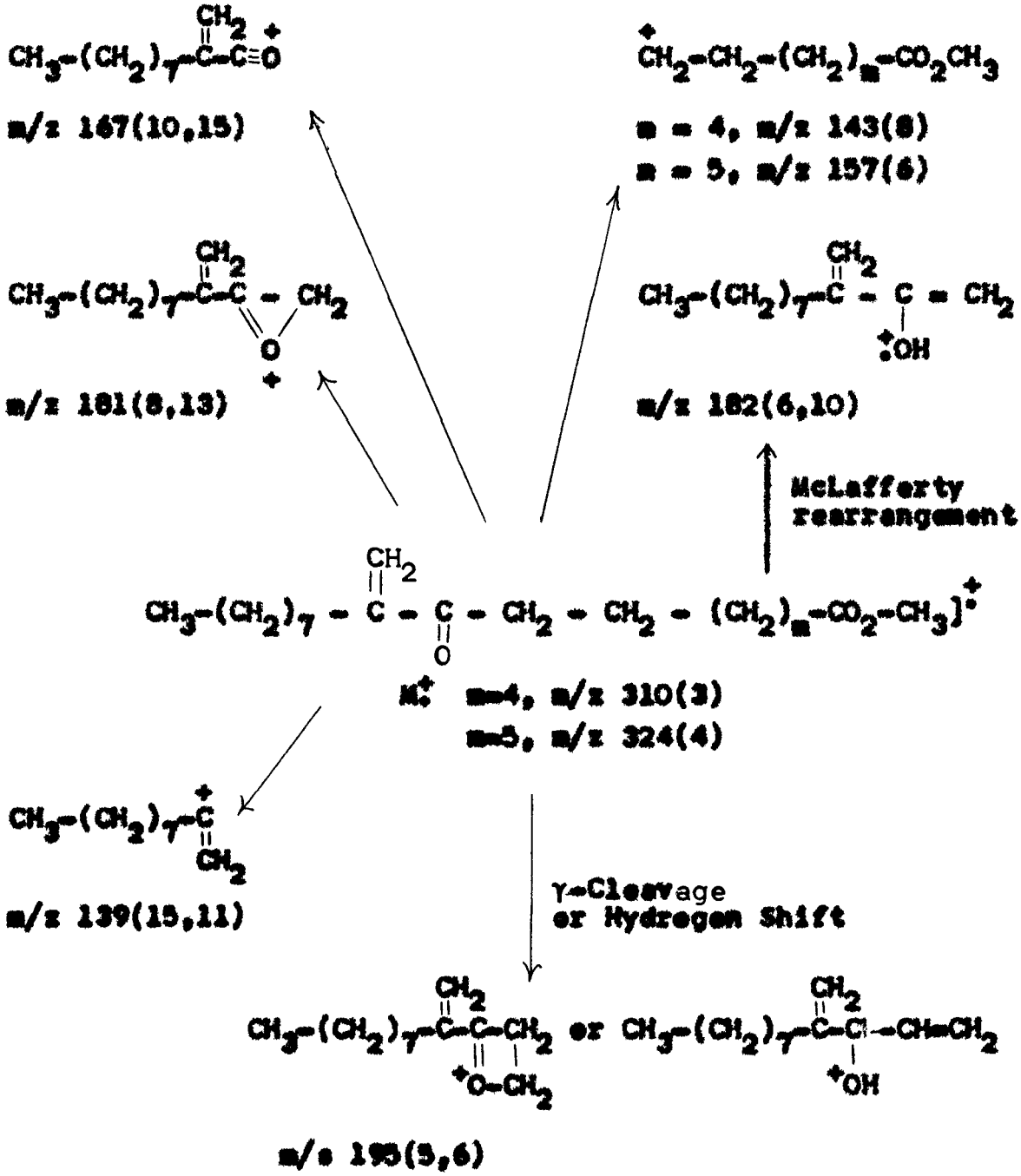


The fragmentation pattern for (XI, XIII) is same as described above.

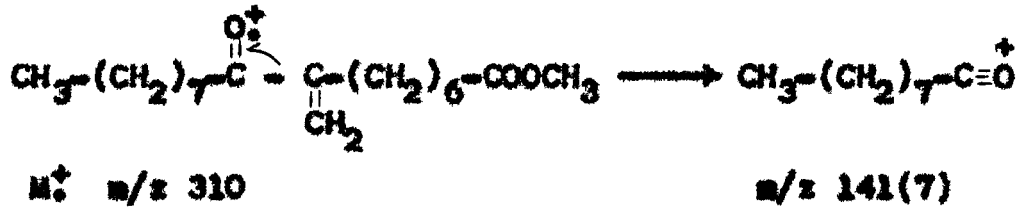
MS of Ketone Derivatives (XIV-XVII)

The mass spectrum of methyl 9(8)-methylene-8(9)-exoheptadecanoate (XIV,XV) and methyl 10(9)-methylene-9(10)-exooctadecanoate (XVI, XVII) gave molecular ion peaks at m/z 310($C_{19}H_{34}O_3$) and 324($C_{20}H_{36}O_3$). Cleavage on either side of the exo group in these derivatives further supported the position of the cyclopropene ring in the fatty ester chain. The usual fragmentation pattern of exo fatty acid was observed. The diagnostic peaks are shown in scheme 6. The peaks at m/z 143, 167 and 139 for 8-exo derivative (XIV), arise by the prefered α-cleavages to exo group. These fragment ions confirmed that three-membered cyclopropene ring was present in the chain at 8,9-position. The isomeric ketone, methyl 8-methylene-9-exoheptadecanoate (XV) gave diagnostic peak at

Scheme 6


m/z 167(10,15)
m = 4, m/z 143(8)
m = 5, m/z 157(6)
m/z 181(8,13)
m/z 182(6,10)
McLafferty rearrangement
M+ m=4, m/z 310(3)
m=5, m/z 324(4)
m/z 139(15,11)
γ-Cleavage or Hydrogen Shift
m/z 195(5,6)

m/z 141. It originates from molecular ion by the cleavage between the α,β-unsaturated ketone. This ion further supports that the cyclopropene ring is between C_8 and C_9 position (methyl malvalate).



$M^{+\cdot}$ m/z 310 m/z 141(7)

In the mass spectrum of 10-methylene-9-oxooctadecanoate (XVI), the most intense peaks at m/z 167 and 157 again established that cyclopropene ring was at 9,10-position (methyl sterculate).

In conclusion it may be mentioned that GLC of silver nitrate treated CPFA is a suitable method for the quantitation of mixed methyl esters containing CPFA and GC-MS study is more reliable for characterization of individual (malvalic and/or sterculic) acids.

1.2. Palmitoleic Acid in *Ochna arteprurpuria* Seed Oil

C_{16}-Monounsaturated acids are not as common in seed fats as those of C_{18} chain length. Among the C_{16}-acids the *cis*/*trans*-unsaturation has been reported at 11, 9, 7, 6, 5 and 3-positions[102,129]; these hexadecenoic acids usually are found in small quantities in seed fats. Spencer *et al.*[130] have reported the presence of *cis*-6-hexadecenoic acid (82.2%) in the seed oil of *Thunbergia alata*. Recently *cis*-9-hexadecenoic acid have been reported from *Roureopsis obliquifoliata*[131] (Connaraceae, 32%) and *Zanthoxylum alatum*[132] (Rutaceae, 15.4%) seed oils.

Previous work[133] on two species of Ochnaceae revealed the presence of higher C_{22} and C_{24} acids, as major components. More interestingly, oil of *Ochna squarrosa*[134] was shown to be a richest source of palmitic acid (73.5%) and tripalmitin (50%). The relative rareness of palmitoleic acid in plants[135], its unusual abundance in *O. arteprurpuria* and paucity of literature on Ochnaceae seed oils led to the compositional study of this additional *Ochna* species.

Exhaustive extraction of the ground seeds of *O. arteprurpuria* with petroleum-ether (40-60C) gave an oil (16.8%). Oil was subsequently neutralized by passing it in chloroform through a short column of alumina to remove the

free fatty acids. Seed and oils properties determined by AOCS methods[1], are summarized in table 3. UV and IR spectral analyses of the oil and its methyl ester showed no conjugation/*trans*-unsaturation or any other unusual functional group. Qualitative TLC indicated the presence of only usual fatty acids. Argentation TLC (Pet. ether:ether, 92:8; v/v) of the methyl esters gave clear spots corresponding to the saturates, monoene and diene.

Table 3

Analytical Data of *Q. artopurpuria*

Oil %	16.8
Moisture %	3.9
Protein content, Nx6.25%	18.5
Refractive index, n_D^{25}	1.4671
Iodine value (Wijs)	78.85
Saponification value	209.9
Ultraviolet (UV)	Usual
Infrared (IR)	Usual

GLC analysis of the original methyl ester of the oil from *Q. artopurpuria* seed oil with the use of the non-polar SE-30 column showed C_{16} and C_{18} fatty acids to be the major components. GLC analysis of methyl ester with the use

of DEGS column showed five well defined peaks in the chromatogram. Tentative identification of these peaks was made by comparison of their relative retention times with those of known fatty acid esters (Table 4).

Table 4

Peak No.	Q. artocarpurea retention time relative to 16:0	Fatty acid	%
1	3.1	16:0	34.1
2	3.5	16:1	25.6
3	5.3	18:0	0.4
4	6.3	18:1	20.3
5	7.4	18:2	19.6

A sample of the methyl ester was separated into three fractions by Ag^{+}/column chromatography. The composition of each fraction is shown in table 5.

Fraction I (34.2% by weight) was analysed by GLC and found to be saturated esters (Table 5). No absorption of hydrogen took place. The palmitic and stearic acids were identified by their emergence times under GLC with those of standard methyl esters.

Table 5

Fatty acid composition of O. artopurpuria methyl ester separated by Ag^+/column chromatography.

Fractions	Peak number from table 4	Fatty acid composition (%) by GLC
I (saturated)	1,3	16:0 (98.8) 18:0 (1.2)
II (Monoene)	2,4	16:1 (55.8) 18:1 (44.2)
III (Diene)	5	18:2 (100)

Fraction II (45.8% by weight) showed the presence of 16:1 (55.8%) and 18:1 (44.2%) acids. No indication of trans-unsaturation was found by IR analysis of the monoene esters.

The position of the double bond in fraction II was established by permanganate-periodate cleavage[136] (Scheme 5). The acidic products were recovered and esterified by usual procedure and the methyl esters were examined by GLC using DEGS column. The C_7 and C_9 monocarboxyl (MC) acids were equivalent to the proportion of 16:1 and 18:1 acids. It is apparent that the heptanoic acid (C_7, XIXa) came from oxidation of the hexadecenoic acid. It has already shown by GLC

retention time that C_{18} monoene has double bond between C_9-C_{10}, which on splitting gave C_9MC(nonanoic) acid (XIXb). The only dicarboxylic acid detected was azelaic acid (XX), the proportion being double the content of monocarboxylic acid. These oxidative cleavage data established the presence of cis-9-hexadecenoic and cis-9-octadecenoic acids in ester fraction II.

Scheme 3

$$CH_3-(CH_2)_n-\overset{9}{CH}=\overset{}{CH}-(CH_2)_7-COOCH_3$$

(XVIIIa,b)

$$\downarrow \; MnO_4^-/IO_4^-$$

$$CH_3-(CH_2)_n-COOH + HOOC-(CH_2)_7-COOH$$

(XIXa,b) (XX)

XVIIIa n = 5; methyl cis-9-hexadecenoate

XVIIIb n = 7; methyl cis-9-octadecenoate

XIXa n = 5; heptanoic acid

XIXb n = 7; nonanoic acid

The diene fraction III (19.3% by weight) has the same retention time as that of standard sample of linoleic acid. IR absorption showed no trans-unsaturation. Oxidative cleavage products by GLC analysis showed the presence of C_6 mono- and C_9 di-carboxylic acids. Thus this fraction was identified as cis-9,12-octadecadienoic (Linoleic) acid.

O. artopurpuria is an unusual acid-containing seed oil, rich in cis-9-hexadecenoic (25.6%). This observation in conjunction with earlier work on Ochnaceae oils suggests that the family Ochnaceae is chemotaxonomically an interesting one elaborating a C_{16} acid as a major component of triglycerides.

Experimental Procedures

(i) Sources of Oilseeds

The seed samples for the present study were obtained by staff botanists from wild plants, under contract in various parts of the country or by purchase from commercial seed suppliers.

(ii) Oil Extraction

Cleaned and dried samples of seed were usually ground in a disintegrator. The powdered seeds extracted repeatedly with light petroleum ether (40-60C) in a Soxhlet apparatus. The extracted oils were dried over anhydrous sodium sulphate. The solvent was removed under reduced pressure. The oil characteristics and seed properties were determined according to the procedure recommended by AOCS[1].

(iii) Preparation of Mixed Fatty Acids

Seed oil was refluxed with ethanolic potassium hydroxide. The unsaponifiable material was removed by ether extraction and the free fatty acids were obtained by acidification of aqueous layer and extracted with ether.

(iv) Methyl Esters

Esterification was carried out as follows: Samples were refluxed for 1 hr. in a large excess of absolute methanol containing 1% sulphuric acid (v/v). The resulting mixtures were diluted to the cloud point with water, chilled in ice bath, and then extracted repeatedly with ether. Combined extracts were washed with $NaHCO_3$ and dried over anhydrous sodium sulphate and evaporated *in vacuo*. In the case of *S. colorata* and *S. foetida* seed oils the methyl esters were prepared by trans-esterification with sodium methoxide (0.5N).

(v) Thin Layer Chromatography (TLC)

(a) Direct TLC

Analytical TLC was performed on plates coated with 0.25 mm or 1.0 mm thick layer of silica gel. The plates were developed with a mixture of pet. ether (40-60): diethyl ether (4:1, v/v). Spots were detected by heat charring after spraying with 20% aqueous solution of perchloric acid.

(b) Reversed-Phase TLC

Reversed-phase TLC of the esters were done on silicenised silica gel plates using acetonitrile:acetic acid:water

(70:10:20; v/v). Spraying of the chromateplates was done with 20% aqueous perchloric acid solution.

(c) Argentation TLC

Silica gel plates impregnated with 20% silver nitrate was used for argentation TLC. Solvent system pet. ether:ether (92:8; v/v) was used for developing the plates.

(vi) Halphen Colour Test[72]

A solution of sulphur (1 g sulphur dissolved in 100 mL CS_2) was prepared for Halphen test. One mL of filtered and dried oil was taken in amyl alcohol (1 mL) and mixed with above reagent (1 mL). The mixture was heated on water bath (70-80C) for a few minutes till carbon disulphide (CS_2) has boiled off. On keeping the test tube in an oil bath (110-115C) for 1-2 hr, a red colour developed which inferred the presence of CPFA.

(vii) HBr-titration of the Oil[77]

The quantitation of total cyclopropenoid material was carried out by titration of weighed amount of the oil with 0.1N hydrogen bromide solution using crystal violet as an indicator at 35C to a bluish green end point, that persists for 30 seconds. The percentage of CPFA content was calculated

by the empirical equation :

$$\% \text{ CPFA} = \frac{29.45 \times N \times V}{\text{Weight of the sample}}$$

where N = normality; V = volume of HBr consumed in titration.

(viii) Preparation of Silver Nitrate-Derivatives[83]

A 200 mg portion of methyl esters of S. colorata oil was treated with absolute methanol (60 ml) saturated with silver nitrate. The reaction was allowed to proceed at room temperature with stirring for 24 hr. The normal methyl esters and the reaction products of the cyclopropenes were recovered from the reaction mixture by adding 100 mL distilled water and extracting with ether. The combined ether extracts were dried over anhydrous sodium sulphate and evaporated in a stream of nitrogen. The freshly prepared methyl esters of S. foetida were also treated with silver nitrate-methanol as above. The esters containing sterculate and malvalate derivatives, thus obtained were used in GC-MS analysis as reference standards. GC data is given in table 2.

(ix) Infrared (IR) Spectra

IR spectra were recorded on Perkin Elmer Model 621 spectrophotometer as liquid film or as 1% solution in carbon tetrachloride.

(x) Ultraviolet (UV) Spectra

UV spectra of oils were recorded on DK-2 ultraviolet spectrometer in methanol.

(xi) Nuclear Magnetic Resonance (NMR) Spectra

NMR spectra were obtained with a Varian A60 spectrometer in CCl_4, the chemical shifts being obtained using tetramethylsilane as the internal reference.

(xii) Gas-liquid Chromatography (GLC)

The quantitative examination of methyl esters were undertaken by using Perkin-Elmer-154, Vapour-fractometer using a 2'x3/16" column of silicone (SE-30, 2%) and 8' x3/16" column of polyester (diethyleneglycol succinate, 15% on chromosorb W, 45-60 mesh). Temperature at the injection port, detector block and column were 290C, 260 and 190C, respectively. Attenuation 4, bridge current 150 m amp. and chart speed 30"/hr., Hydrogen flow rate 70 mL/min.

Linseed oil methyl esters and silver nitrate treated methyl esters of Sterculia foetida seed oil were used as reference standard.

(xiii) GC-MS Analysis

In GC-MS, the instrument used was Pye model 104 gas chromatograph fitted with a split effluent (ratio 1:3) to (a) a flame ionization detector and (b) a silicone rubber membrane separated into a modified fast scanning AEI MS9 mass spectrometer. The glass column (1.8 m x 2 mm, i.d.) was packed with polyethyleneglycol adipate (PEGA, 5%) on 100/120 mesh chromosorb G-AWDMCS. The column oven was 185C, and helium flow was 40 mL/min. The peaks were analyzed using a data system consisting of an Inotem Maxi data system, with a carrick interface and a P.D.P11D minicomputer and tele type.

Identification of the component acids was made by comparing retention time with that of reference standard. Peak areas, calculated from peak height and width of half height, are reported as composition (% wt.) in table 2.

Quantitative Separation of Mixed Methyl Esters of O. artopurpuria Seed Oil by Ag^+/Column[137]

The mixed methyl esters of O. artopurpuria seed oil was separated into saturates, monoene and diene using Ag^+/column. 3.0 g ester were fractionated over Ag^+/column (40 g silica gel) impregnated with silver nitrate (17 g) using pet. ether-ether as moving phase. During the chromatographic runs

5-8 mL fraction were collected. Elution with pet. ether gave saturated fraction I (1.02 g). Subsequent elution with pet. ether-ether (99.5:0.5, v/v) gave fraction II (1.35 g). Final elution with pet. ether-ether (99:1, v/v) afforded fraction III (0.58 g). Each fraction was analyzed by GLC and composition is given in table 5.

Permanganate-Periodate Oxidation[136] of Fractions II and III

Fraction II (0.2 g) was stirred 4 hr. with sodium periodate (1.60 g), potassium permanganate (0.04 g) and potassium carbonate (0.76 g) in water (140 mL). The reaction was terminated by addition of excess of sodium bisulphite. The mixture was acidified with dil. sulphuric acid and extracted repeatedly with ether. Combined ethereal portion was washed with water and dried over sodium sulphate. The products obtained were esterified with diazomethane and subjected to GLC. A portion of products was crystallized from pet. ether to get dicarboxylic acid (m.p. 106-107C). The dicarboxylic acid after esterification was subjected to GLC and showed the same retention time as that of azelaic acid ester.

Fraction III was treated with permanganate-periodate as described above.

2.1. Glyceride Structure of the Seed Oil of *Ochna squarrosa* (Ochnaceae)

A recent report[134] from author's laboratory showed that the seed oil of *O. squarrosa* is a very unusual oil in having a high content of monoacid triglyceride (Tripalmitin, 50%). The oil's potential usefulness as a source of industrial palmitic acid led to a study of its glyceride structure. The data obtained from pancreatic lipase hydrolysis was used in the determination of glyceride structure of this oil.

The glyceride structure was determined on the whole neutral oil. The reaction procedure was based on the semi-micro method developed by Luddy *et al.*[93a] The fatty acid composition of the triglycerides and that of the 2-monoglycerides are given in table 6. The apparent percentage of each acid in 1,3-position was calculated by the difference from the monoglyceride and triglyceride[138].

According to the theory of Gunstone[100,102] the 2-position of the glycerol moiety is preferentially acylated by C_{18} unsaturated acids and there is a preference for linoleic acid over oleic and linolenic acids for 2-position. It will be evident from table 6 that the present findings agree closely though not completely with the hypothesis for the acylation of the 2-position by C_{18} unsaturated acids. The

monoglyceride of seed oil of Q. squarrosa as obtained by lipolysis is composed of 35.9 mole percent of C_{18} unsaturated acids. The linoleic acid doesnot show the preference over oleic acid. This deviation may be due to the presence of high amount of palmitic acid.

Table 6

Fatty acids in glyceride positions of Q. squarrosa (mole %)

Fatty acids		P 16:0	St 18:0	O 18:1	L 18:2
(a)	Fatty acid composition of triglyceride (TG)	70.3	1.6	15.8	12.3
	(a) x 3	210.9	4.8	47.4	36.9
(b)	Fatty acid composition of 2-monoglycerides (2-MG)	64.1	-	20.3	15.6
	(a) x 3 - b	146.8	4.8	27.1	21.3
(c)	Fatty acid in 1,3-position	73.4	2.4	13.6	10.6
	$\frac{(a) \times 3 - b}{2}$				

The enrichment factors[100,139] for palmitic, oleic and linoleic acids are summarized in table 7. The enrichment factors for oleic and linoleic acids (1.28 and 1.27×, respectively) are almost same and preferentially acylated to 2-position of the glycerol moiety. A high enrichment factor (0.91) for palmitic acid shows that it is also acylated to 2-position.

Table 7

Fatty acid distribution in 2-monoglycerides (2-MG) from *Q. squarrosa* oil.

Fatty acids	Mole Percent	Enrichment[a] Factor, E	Molar Percentage[b] of acid at 2-position
16:0	64.1	0.91	30.4
18:0	-	-	-
18:1	20.3	1.28	42.82
18:2	15.6	1.27	42.3

a. Enrichment factor, E = $\frac{\text{mole percent acid at 2-position of TG}}{\text{mole percent of same in total TG}}$

b. Molar percent acid at 2-position = $\frac{\text{mole percent acid in 2-MG}}{3 \times \text{mole percent of same in TG}} \times 100$

Triglyceride composition of Q. squarrosa seed oil was calculated from the fatty acid composition of the neutral triglyceride and the corresponding 2-monoglycerides using the calculation of Vander Wal[97] and Coleman[138]. The glycerides of individual fatty acids of this oil are listed in table 8.

Table 8

Glycerides of individual fatty acids in Q. squarrosa*

Palmitic (P)		Oleic (O)		Linoleic (L)	
Glycerides	Mole Percent	Glycerides	Mole Percent	Glycerides	Mole Percent
PPP	34.53	POP	10.93	PLP	8.40
PPSt	2.26	POSt	0.72	PLSt	0.54
PPO	12.80	POO	4.04	PLO	3.12
PPL	9.98	POL	3.16	PLL	2.42
StPSt	0.04	StOSt	0.01	StLSt	0.01
StPO	0.42	StOO	0.14	StLO	0.10
StPL	0.33	StOL	0.10	StLL	0.08
OPO	1.18	OOO	0.38	OLO	0.29
OPL	1.85	OOL	0.59	OLL	0.45
LPL	0.72	LOL	0.23	LLL	0.18
	64.11		20.30		15.59

* The glycerides of stearic acid (St) comes out to be zero.

Table 9

Composition of Triglycerides of Q. squarrosa

Triglycerides	Mole Percent
GS_3	36.83
GS_2U	44.14
GSU_2	16.91
GU_3	2.12

S = saturated fatty acids, U = unsaturated fatty acids.

Ali et al.[134] have reported that Q. squarrosa seed oil contains an exceptionally high amount of tripalmitin (30% by weight). A maximum of 34.53 mole percent tripalmitin was predicted by using 1,3-random-2-random distribution pattern. The composition of glycerides is given in table 9. Only small amount (2.12 mole percent) of triunsaturated glycerides is present. The abundance of saturated triglyceride reveals the commercial importance of this seed oil in industry.

2.2. Glyceride Structure of the Seed Oils of *Ochna arteneurouria* and *Zanthoxylum alatum*

In the earlier section, the fatty acid composition of *O. arteneurouria* has been worked out by the GLC. The unusual palmitoleic acid (25.6%) was found as one of the components of this seed oil. *Zanthoxylum alatum*, Roxb. (Rutaceae) seed oil was analyzed for fatty acid composition by Ahmad *et al.*[132] who found the presence of 15.4% palmitoleic (*cis*-9-hexadecenoic) acid. The high contents of palmitoleic acid in these two seed oils, prompted the author to study the distribution of this acid in the triglycerides.

The purified triglycerides were prepared by eluting the oils over activated Florisil[140] and hydrolyzed by pancreatic lipase[93a]. The fatty acid composition of the original triglycerides and monoglycerides produced by lipase hydrolysis is given in table 10. The fatty acid composition in 1,3-position was calculated by the difference from the monoglycerides and triglycerides.

Within the limits of experimental error, palmitic and stearic acids are exclusively distributed at the 1,3-position in *O. arteneurouria* and *Z. alatum*. The palmitoleic acid represented 46.10% of the fatty acids at the 2-position of the glycerol in *O. arteneurouria*. In *Z. alatum*, palmito-

leic and oleic acids appeared to be almost equally distributed between the 1,3- and 2-positions and linolenic acid preferentially acylated at 2-position (Table 10). The distribution of palmitoleic acid in Z. alatum is in agreement with the findings of Raju[141] in the distribution of cis-9-hexadecenoic acid in Tricuspidaria lanceolata (Elaeocarpaceae) and those of Gunstone et al.[102] in Macadamia ternifolia seed oil or the cis-11-hexadecenoic acid in Genuina avellana seed oil.

Table 10

Fatty acids in glyceride portions of Q. artopurpuria and Z. alatum.

Seed Fat		Fatty acids (mole %)					
		16:0	16:1	18:0	18:1	18:2	18:3
Q. artopurpuria	TG	34.1	25.6	0.4	20.3	19.6	-
	2-MG	0.7	46.10	-	25.53	27.66	-
	1, 3	50.8	15.35	0.6	17.68	15.57	-
Z. alatum	TG	19.5	15.6	2.3	22.8	19.2	20.5
	2-MG	0.3	15.2	-	21.74	25.78	36.95
	1, 3	29.1	15.8	3.45	23.33	15.91	12.27

The enrichment factor and molar percentage of acid at 2-position are given in table 11. It was inferred from

the table 11 that the order of preference for 2-position in *O. artemisiifolia* is 16:1 > 18:2 > 18:1 while in *Z. alatum* it is 18:3 > 18:2 > 16:1 > 18:1.

Table 11

Fatty acid distribution in 2-monoglycerides from *O. artemisiifolia* and *Z. alatum*.

Seed Fat		Fatty acids (mole %)					
		16:0	16:1	18:0	18:1	18:2	18:3
O. artemisiifolia	2-MG	0.7	46.10	-	25.53	27.66	-
	E	0.02	1.80	-	1.25	1.41	-
	M	0.68	60.04	-	41.92	47.04	-
Z. alatum	2-MG	0.3	15.2	-	21.74	25.78	36.95
	E	0.015	0.97	-	0.95	1.34	1.80
	M	0.51	32.48	-	31.78	44.75	60.08

E= Enrichment factor; M= Molar percentage of acid at 2-position.

The triglyceride compositions of *O. artemisiifolia* and *Z. alatum* were calculated from fatty acid compositions of neutral oils and of the monoglycerides produced from it by pancreatic lipase hydrolysis using the procedure of Vander Wal[97] and Coleman[138]. The pattern of distribution of glycerides is shown in table 12. The positional isomers of tri-

glycerides less than 0.1 mole percent are not shown in table 12. The glyceride types in both oils worked out to be :

	Q. artopurpuria	Z. alatum
Trisaturated glyceride (GS_3)	0.18	0.04
Disaturated glyceride (GS_2U)	26.59	10.63
Monosaturated glyceride (GSU_2)	48.51	43.93
Triunsaturated glyceride (GU_3)	24.58	44.97

Coleman's procedure of calculation is helpful in computing the glyceride structure of whole oil. The determination of glyceride structures of Q. artopurpuria and Z. alatum oils, following the lipolysis cum GLC technique showed the glyceride contents that are anticipated from the composition of their component fatty acids.

Table 12

Composition of triglycerides of Q. artopurpuria and Z. alatum.

Triglyceride class	Positional isomers of triglyceride	Mole percentage Q. artopurpuria	Z. alatum
GS_3	PPP	0.18	0.1
GS_2U	PPO	0.13	0.1
	PPL	0.12	0.1

Contd.

	PPPe	0.11	0.1
	POP	6.59	1.84
	POSt	0.16	0.43
	PPeP	11.89	1.29
	PPeSt	0.28	0.31
	PLP	7.14	2.18
	PLSt	0.16	0.52
	PLiP	-	3.13
	PLiSt	-	0.74
GSU_2	PPeO	8.24	2.06
	PPeL	7.29	1.40
	PPePe	7.19	1.40
	PePeSt	0.1	0.16
	LiPeSt	-	0.13
	POO	4.58	2.90
	POPe	1.99	1.97
	OOSt	0.1	0.35
	POL	4.04	1.98
	StOL	0.1	0.24
	PeOSt	0.1	0.24
	LiOSt	-	0.18
	POLi	-	1.53
GSU_2	PLL	4.38	2.38
	PLO	4.97	3.25
	PLPe	4.32	2.36
	StLL	0.1	0.28
	StLPe	0.1	0.28
	StLO	0.1	0.40
	LiLSt	-	0.22
	LiLP	-	1.84
	StLiO	-	0.60
	LLiSt	-	0.41

Contd.

	PLiL	-	3.42
	PLiO	-	5.01
	PLiLi	-	2.64
	PoLiP	-	3.40
	PoLiSt	-	0.40
	LiLiSt	-	0.31
GU_3	PoPoPo	1.09	0.38
	PoPoO	2.50	0.56
	PoPoL	2.20	0.76
	PoPoLi	-	0.59
	OPoO	1.44	0.83
	OPoLi	-	0.87
	OPoL	2.54	1.13
	LiPoLi	-	0.23
	LiPoL	-	0.59
	OOO	0.80	1.18
	OOPo	1.39	1.60
	OOL	1.41	1.61
	OOLi	-	1.24
	PoOLi	-	0.84
	LiOL	-	0.85
	LOL	0.62	0.35
	LOPo	1.22	1.10
	LiOLi	-	0.33
	PoOPo	0.60	0.34
	LLL	0.67	0.68
	LLO	1.32	1.91
	LLPo	1.32	1.29
	LLLi	-	1.00
	OLO	0.86	1.40
	PoLPo	0.65	0.64
	LiLPo	-	1.0

Contd.

OLPo	2.75	1.90
OLLi	-	1.48
LiLLi	-	0.39
LiLiLi	-	0.56
LiLiL	-	1.44
LiLiO	-	2.12
LiLiPo	-	1.43
PoLiPo	-	0.92
PoLiL	-	1.86
PoLiO	-	2.72
OLiL	-	1.02
OLiO	-	2.01
LLiL	-	0.93

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* The positional isomers of triglycerides less than 0.1 mole percent are not shown in table.

P = palmitic, Po = Palmitoleic, O = Oleic, L = Linoleic and Li= Linolenic.

Experimental Procedures

The extraction of oils and preparation of esters have been detailed in chapter 1. Prior to lipolysis, the oils (2.0 g) were neutralized by passing over activated Florisil (18 g) using benzene (400 mL) as eluting solvent.

Pancreatic Lipase Hydrolysis of Triglycerides

The reaction procedure was based on the semimicro method developed by Luddy *et al.*[93a] To the neutral triglycerides (50 mg) weighed in a small centrifuge tube, was added pancreatic lipase (9 mg). Then 1 mL of 1M tris-hydroxymethylaminomethane (tris-buffer) at pH 8.0, 0.1 mL 22% calcium chloride solution and 0.25 mL of 0.1% bile salt solution were quickly added. After keeping the reaction mixture at 40C for 1 minute, it was stirred at this temperature for 8 minutes.

Lipolysis was terminated by acidification with 0.5 mL hydrochloric acid (1:1). The reaction mixture was then immediately extracted three times with ether. The ether solution was washed with water and dried over sodium sulphate. Solvent was removed under reduce pressure.

Isolation of Lipolytic Products

The lipolytic products were separated by preparative TLC on 20 x 20 cm plates spread with a 1 mm layer of silica gel. The slurry was prepared with saturated solution of boric acid. The lipolytic products were dissolved in chloroform to get 1:10 dilution and the solution was applied as a band on preparative TLC. Two to three chromatoplates were used. The plates were developed with hexane-diethyl-ether-acetic acid (80:20:1, v/v). Bands were detected under UV light with 2',7'-dichlorofluorescein as the fluorescent indicator. The components separated into four well-defined zones in the following order of increasing height travelled: monoglycerides, diglycerides, free fatty acid and unreacted glycerides. The 2-monoglycerides (2-MG) portion was scraped off, extracted three times with diethylether, converted into methyl esters by transesterification using 0.4 percent sodium methoxide in anhydrous methanol.

Gas-liquid Chromatography (GLC)

DEGS column was used as described in previous chapter. The separations were carried out isothermally at 200C, chart speed 30 inch h^{-1} with a helium flow of 30 mL $minute^{-1}$.

The glyceride structure was also determined using pancreatic lipase hydrolysis data and applying Vander Wal[97] and Coleman[138] method of calculation.

References

1. Official and Tentative Methods of American Oil Chemists' Society , AOCS, Champaign, Illinois, 3rd edition, 1 (1971).

2. Smith, Jr., C.R., T.L. Wilson, E.H. Melvin and I.A. Wolff, J. Am. Chem. Soc. 82:1417 (1960).

3. Binder, R.G., T.H. Applewhite, M.J. Diamond and L.A. Goldblatt, JAOCS. 41:108 (1964).

4. Morris, L.J., R.T. Holman and K. Fontell, ibid. 37: 323 (1960).

5. Chisholm, M.J. and C.Y. Hopkins, Can. J. Chem. 38: 2500 (1960).

6. Tallent, W.H., J. Harris, I.A. Wolff and R.E. Lundin, Tetrahedron Lett. 4329 (1966).

7. Badami, R.C. and L.J. Morris, JAOCS. 42:1119 (1965).

8. Powell, R.G., C.R. Smith, Jr. and I.A. Wolff, J. Org. Chem. 32:1442 (1967).

9a. Phillips, B.E. and C.R. Smith, Jr., Biochem. Biophys. Acta 218:71 (1970).

b. Phillips, B.E., C.R. Smith, Jr. and L.W. Tjarks, ibid. 210:353 (1970); J. Org. Chem. 35:1916 (1970).

10. Kreween, C.F., JAOCS. 45:250 (1968).

11. Earle, F.R., ibid. 47:510 (1970).

12. Gunstone, F.D., J. Chem. Soc. 1611 (1954).

13. Smith, Jr., C.R., Prog. Chem. Fats and other Lipids (ed. R.T. Holman) 11:137 (1970).

14. Kleiman, R., G.F. Spencer, L.W. Tjarks and F.R. Earle, Lipids 6:617 (1971).

15. Kulkarni, S.B. and V.V.S. Mani, J. Oil Tech. Assn. (India) 11:67 (1979).

16. Yazicioglu, T., A. Karaali and J. Gokcen, JAOCS. 55: 412 (1978).

17. Spencer, G.F., Phytochem. 16:282 (1977).

18. Kleiman, R., R.D. Plattner and G.F. Spencer, Lipids 12:610 (1977).

19. Conacher, H.B.S. and F.D. Gunstone, ibid. 5:137 (1970).

20. Ulchenko, N.T., E.I. Gigienova, A.U. Umarov, Khim. Prir. Soedin 4:514 (1978).

21. Husain, S.K. Ph.D. Thesis , Aligarh Muslim University, Aligarh (1978).

22. Hasan, S.Q., M.R.K. Sherwani, I. Ahmad, F. Ahmad and S.M. Osman, J. Ind. Chem. Soc. 57:920 (1980).

23. Ahmad, M.U., S.K. Husain and S.M. Osman, J. Sci. Food Agric. 29:372 (1978).

24. Husain, S.R., M.S. Ahmad, Jr., F. Ahmad and S.M. Osman, Fette Seifen. Anstrichm. (In Press).

25. Siddiqi, S.F., F. Ahmad, M.S. Siddiqi, S.M. Osman and G.R. Fenwick, JAOCS. (In Press).

26. Hashmi, M., M. Khan, M.S. Ahmad, Jr., S. Ahmad and S.M. Osman, J. Oil Tech. Assn.(India) 14:64 (1982).

27. Riser, G.R., J.H. Hunter, J.S. Ard and L.P. Witnauer, JAOCS. 39:266 (1962).

28. Swern, D., R. Weider, M. McDonough, D.R. Meranze and M.B. Shimkin, Cancer Res. 30:1037 (1970).

29. Carter, F.L. and V.L. Frampton, Chem. Rev. 64:497 (1964).

30. Recourt, J.H., G. Jurriens and M. Schinitz, J. Chromateg. 30:35 (1967).

31. Christie, W.W. Topics in Lipid Chemistry (ed. F.D. Gunstone), Legos Press Ltd. London, p.1 (1970).

32. Phelps, R.A., F.S. Shenstone, A.R. Kemmerer and R.J. Evans, Poult. Sci. 44:358 (1965).

33. Vickery, J.R., JAOCS. 57:87 (1980).

34. Morris, L.J. and S.W. Hall, Chem. Ind.(London) 32 (1967).

35. Bohannon, M.B. and R. Kleiman, Lipids 13:270 (1978).

36. Jevans, A.W. and C.Y. Hopkins, Tetrahedron Lett. 18: 2167 (1968).

37. Badami, R.C., K.B. Patil, Y.V. Subbarao, G.S.R. Sastri and G.K. Vishvanathrao, Fette Seifen. Anstrichm. 82:317 (1980).

38. Raju, P.K. and R. Reiser, Lipids 1:10 (1966).

39. Johnson, A.R., J.A. Pearson, F.S. Shenstone, A.C. Fogerty and J. Giovanelli, ibid. 2:308 (1967).

40. Ackman, R.G. and S.N. Hooper, JAOCS. 47:525 (1970).

41. Berry, S.K., ibid. 59:57 (1982).

42. Idem., J. Food Sci. Tech. 17:224 (1980).

43. Kittur, M.H., C.S. Mahajan, K.V.S.A. Rao and G. Lakshminarayana, JAOCS. 59:123 (1982).

44. Ralaimanarivo, A., E.M. Gaydou and J.P. Bianchini, Lipids 17:1 (1982).

45. Madrigal, R.V. and C.R. Smith, Jr., ibid. 8:407 (1973).

46. Ahmad, M.U., S.K. Hussain, M. Ahmad, S.M. Osman and R. Subbarao, JAOCS. 53:698 (1976).

47. Ahmad, Jr., M.S., M.U. Ahmad, S.M. Osman and J.A. Ballentine, Chem. Phys. Lipids 25:29 (1979).

48. Ahmad, M.U., S.K. Hussain, I. Ahmad and S.M. Osman, J. Sci. Food Agric. 30:424 (1979).

49. Hussain, S., M. Babu, M.U. Ahmad, A.A. Ansari and S.M. Osman, Fette Seifen. Anstrichm. 82:29 (1980).

50. Siddiqi, I.A., S.M. Osman, M.R. Subbaram and K.T. Achaya, Ind.J. Chem. 9:209 (1971).

51. Qazi, G.A., S.M. Osman and M.R. Subbaram, J. Oil Tech. Assn.(India) 6:16 (1974).

52. Babu, M., S. Husain, M.U. Ahmad and S.M. Osman, Fette Seifen. Anstrichm. 82:63 (1980).

53. Sinha, S. and S.M. Osman, J. Sci. Food Agric.(In Press).

54. Lie Ken Jie, M.S.F. and M.F. Chan, J. Chem. Soc., Chem. Commun. 3:78 (1977).

55. Kleiman, R., F.R. Earle and I.A. Wolff, Lipids 4:317 (1969).

56. Spencer, G.F., K. Payne-Wahl, R.D. Plattner and R. Kleiman, ibid. 14:72 (1979).

57. Allen, E., A.R. Johnson, A.C. Fogerty, J.A. Pearson and F.S. Shenstone, ibid. 2:419 (1967).

58. Jones, L.A. Antinutrients and Natural Toxicants in Foods (ed. R.L. Ory), Food and Nutrition Press, Inc., Westport, U.S.A., p.77.

59. Miller, A.M., E.T. Sheehan and M.G. Vavich, Proc. Soc. Exp. Biol. Med. 131:61 (1969).

60. Sinnhuber, R.O., D.J. Lee, J.H. Wales and J.L. Ayres, J. Natl. Cancer Inst. 41:1293 (1968).

61. Lee, D.J., J.H. Wales, R.O. Sinnhuber, Cancer Res. 28: 2312 (1968); 31:960 (1971).

62. Sinnhuber, R.O., D.J. Lee, J.H. Wales, M.K. Landers and A.C. Keyl, J. Natl. Cancer Inst. 53:1285 (1974).

63. Sinnhuber, R.O., J.D. Hendricks, G.B. Putnam, J.H. Wales, N.E. Pawloski and J.E. Nixon, Fed. Proc. 35:505 (1976).

64. Loveland, P.M., J.E. Nixon, N.E. Pawloski, T.A. Eisele, L.M. Libbey and R.O. Sinnhuber, J. Environ. Pathol. Toxicol. 2:707 (1979).

65. Selivonchicka, D.P., J.L. Williams and H.W. Schaup, Lipids 16:211 (1981).

66. Shenstone, F.S., J.R. Vickery and A.R. Johnson, J. Agr. Food Chem. 13:410 (1965).

67. Wilson, T.L., C.R. Smith, Jr. and K.L. Mikolajczak, JAOCS. 38:696 (1961).

68. Smith, G.N. and J.D. Bu'Lock, Biochem. Biophys. Res. Commun. 17:433 (1964).

69. Idem., Chem. Ind.(London) 1840 (1965).

70. Hooper, N.K. and J.H. Law, Biochem. Biophys. Res. Commun. 18:426 (1965).

71. Yano, I., L.J. Morris, B.W. Nichols and A.T. James, Lipids 7:35 (1972).

72. Halphen, G., J. Pharm. 6:390 (1897); J. Chem. Soc. 74: 358 (1898).

73. Zahorsky, V. and K.L. Rinehart, Personal Communication Reported by F.L. Carter and V.L. Frampton in Chem. Rev. 64:497 (1964).

74. Deutschman, Jr., A.J. and L.S. Klaus, Anal. Chem. 32: 1809 (1960).

75. Sheehan, E.T., D.L. Schneider and M.G. Vavich, J. Agr. Food Chem. 20:119 (1972).

76. Coleman, E.C. and D. Firestone, J. Assn. Off. Agric. Chem. 55:1288 (1972).

77. Harris, J.A., F.C. Magne and E.L. Skau, JAOCS. 40:718 (1963).

78. Magne, F.C., J.A. Harris, R.A. Pittman and E.L. Skau, ibid. 43:519 (1966).

79. Feuge, R.O., Z. Zarins, J.L. White and R.L. Holmes, ibid. 46:185 (1969).

80. Rosie, D.A. and G.G. Shone, J. Chem. Soc., Perkin Trans. I, 1790 (1972).

81. Feuge, R.O., L.P. Codifer, Jr. and H.J. Zeringue, Jr., JAOCS. 58:718 (1981).

82. Zeringue, Jr., H.J. and R.O. Feuge, ibid. 58:38 (1981).

83. Schneider, E.L., S.P. Lake and D.T. Hopkins, ibid. 45: 585 (1968).

84. Fisher, G.S. and W.H. Schuller, ibid. 58:943 (1981).

85. Coleman, E.C., J. Assn. Off. Agric. Chem. 53:1209(1970).

86a. Miwa, T.K., JAOCS. 40:309 (1963).

b. Gellerman, J.L. and H. Schlenk, Anal. Chem. 38:72 (1966).

87. Kircher, H.W., JAOCS. 41:4 (1964).

88. Idem., ibid. 42:899 (1965).

89. Pawlowski, N.E., J.E. Nixon and R.O. Sinnhuber, ibid. 49:387 (1972).

90. Jurriens, G. and A.C.J. Kroesen, ibid. 42:14 (1965).

91a. Litchfield, C., M. Farquhar and R. Reiser, ibid. 41: 588 (1964).

b. Zadernowski, R. and F. Sosulski, ibid. 56:1004 (1979).

92. Kuksis, A. and W.C. Breckenridge, ibid. 42:978 (1965).

93a. Luddy, F.E., R.A. Barford, S.F. Herb, P. Magidman and R.W. Riemenschneider, ibid. 41:693 (1964).

b. Matteen, F.H. and L.W. Beck, J. Biol. Chem. 219:735 (1956).

94. Litchfield, C., Lipids 3:170 (1968).

95. Greb, Jr., K., H.P. Neukom and R. Battaglia, JAOCS. 57:28 (1980).

96a. Karleskind, A., G. Valmalle, O. Midler and M. Blank, Rev. Fr. Corps. Gras 25:351 (1978).

b. Plattner, R.D. and K. Payne-Wahl, Lipids 14:152 (1979).

97. Vander Wal, R.J., JAOCS. 37:18 (1960).

98. Coleman, M.H. and W.C. Fulton, Enzymes of Lipid Metabolism , Pergamon Press, New York, p.127 (1961).

99. Litchfield, C., Analysis of Triglycerides , Academic Press, New York and London (1972).

100. Gunstone, F.D., Chem. Ind.(London) 1214 (1962).

101. Matteon, F.H. and R.A. Volpenhein, J. Lipid Res. 4: 392 (1963).

102. Gunstone, F.D., R.J. Hamilton, F.B. Padley and M.I. Qureshi, JAOCS. 42:965 (1965).

103. Evans, C.D., D.G. McConnel, G.R. List and C.R. Scholfield, ibid. 46:421 (1969).

104. Litchfield, C., ibid. 48:467 (1971).

105a. Appelqvist, L.A. Rapeseed (ed. L.A. Appelqvist and R. Ohlson), Elsevier Publishing Co. Amsterdam, p.123 (1972).

b. Grynberg, H. and K. Ceglowska, Rev. Fr. Corps. Gras 17:173 (1970).

c. Rietsch, J., ibid. 29:75 (1982).

106. Pee, W.V., L. Boni, M. Fema, M. Hoylaerts and A. Hendrikx, JAOCS. 57:243 (1980).

107. Cole, E.R., G. Crank and A.S. Sheikh, ibid. 57:23 (1980).

108. Lehrian, D.W., P.G. Keeney and D.R. Butler, ibid. 57:66 (1980).

109a. Quedraogo, M. and J. Bezard, Rev. Fr. Corps. Gras 28:473 (1981).

b. Sengupta, A. and S.K. Roy Choudhury, J. Sci. Food Agric. 27:165 (1976).

110. Rajiah, A. and M.R. Subbaram, J. Oil Tech. Assn. (India) 6:13 (1974).

111. Osman, F., M.R. Subbaram and K.T. Achaya, Fette Seifen. Anstrichm. 70:69 (1968).

112. Quedraogo, M.A. and J.A. Bezard, Rev. Fr. Corps. Gras 29:11 (1982).

113. Sengupta, A. and S.K. Roy Choudhury, JAOCS. 55:621 (1978).

114. Fatemi, S.H. and E.G. Hammond, Lipids 12:1032 (1977).

115. Strocchi, A., J. Food Science 47:36 (1981).

116. Sengupta, A. and U.K. Mazumdar, JAOCS. 53:680 (1976).

117. Lognay, G., A. Ergo, J.P. Wathelet and M. Severin, Rev. Fr. Corps. Gras 28:67 (1981).

118. Kleiman, R., G.F. Spencer, F.R. Earle, H.J. Nieschlag and A.S. Barclay, Lipids 7:660 (1972).

119. Mikolajczk, K.L. and C.R. Smith, Jr., Biochem. Biophys. Acta 152:244 (1968).

120. Plattner, R.D., K. Payne-Wahl, L.W. Tjarks and R. Kleiman, Lipids 14:576 (1979).

121. Rajiah, A., M.R. Subbaram and K.T. Achaya, Lipids 11: 82 (1976).

122. Madrigal, R.V. and C.R. Smith, Jr., Lipids 17:650 (1982).

123. Smith, Jr., C.R., R.V. Madrigal, D. Weisleder and R.D. Plattner, ibid. 12:736 (1977).

124. Miller, R.W., C.R. Smith, Jr., D. Weisleder, R. Kleiman and W.K. Rohwedder, ibid. 9:928 (1974).

125. Phillips, B.E., C.R. Smith, Jr. and J.W. Hagermann, ibid. 4:473 (1969).

126. Ansari, F.A., Ph.D. Thesis , Aligarh Muslim University, Aligarh (1972).

127a. Roehn, J.N., D.J. Lee, J.H. Wales, S.D. Pellilyka and R.O. Sinnhuber, Lipids 5:80 (1970).

b. Abou-Asheur, A.M. and H.M. Edwards, J. Nutrition 100: 1374 (1970).

128. Duthie, J.F., Flora of the Upper Gangetic Plain and the Adjacent Sivalik and Sub-Himalayan Tracts, Jayyed Press, Delhi, Part 1, p.101 (1973).

129a. Batty, M.K. and B.M. Craig, Can. J. Biochem. 44:311 (1966).

b. Gunstone, F.D., G.M. Taylor, J.A. Cornelius and T.W. Hammond, J. Sci. Food Agric. 19:706 (1968).

c. Spencer, G.F., R. Kleiman, F.R. Earle and I.A. Wolff, Lipids 5:277 (1970).

d. Raju, P.K. and R. Raymond, JAOCS. 45:583 (1968).

130. Spencer, G.F., R. Kleiman, R.W. Miller and F.R. Earle, Lipids 6:772 (1971).

131. Spencer, G.F. and R. Kleiman, JAOCS. 55:689 (1978).

132. Ahmad, F., I. Ahmad and S.M. Osman, ibid. 57:224 (1980).

133a. Hilditch, T.P., M.L. Meara and C.B. Patel, J. Sci. Food Agric. 2:142 (1951).

b. Hilditch, T.P. and M.L. Meara, J. Soc. Chem. Ind. 63:114 (1944).

134. Ali, M.L., M.S. Ahmad, F. Ahmad and S.M. Osman, Chem. Ind.(London) 237 (1980).

135. Hilditch, T.P. and P.N. Williams, The chemical constitution of Natural Fats , Chapman and Hall, London, p.14 (1964).

136. von Rudloff, E., Can. J. Chem. 34:1413 (1956).

137. De Vries, B., JAOCS. 40:184 (1963).

138. Coleman, M.H., ibid. 38:685 (1961).

139. Gunstone, F.D., R.J. Hamilton and M.I. Qureshi, J. Chem. Soc. 319 (1965).

140. Carella, K.K., J. Lipid Res. 2:135 (1961).

141. Raju, P.K., JAOCS. 45:583 (1968).

142. Daulatabad, C.D. and R.F. Ankalgi, JAOCS. 59:439 (1982).

Part II

Reactions of α,β-Unsaturated Fatty Compounds

Theoretical

Reactions of α,β-Unsaturated Fatty Compounds and their Derivatives

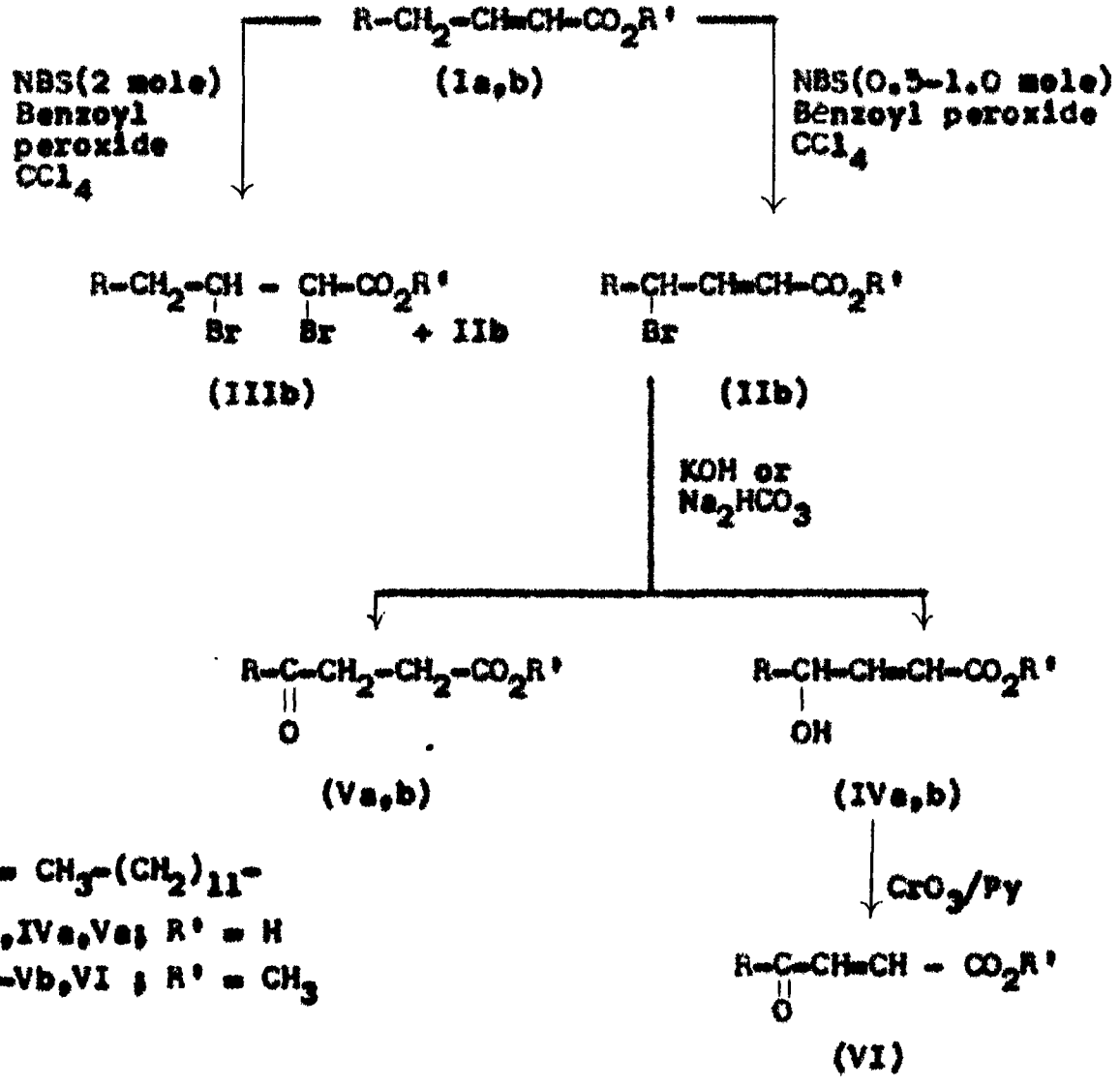
A review of literature reveals that the olefinic fatty compounds and their derivatives so far studied are mostly confined to the acids containing internal double bonds in positions 6,7; 9,10 and 13,14 of the fatty chain. Further the long-chain α,β-unsaturated compounds and their derivatives have not been thoroughly investigated, probably due to their non-availability in natural fats. Some short-chain α,β-unsaturated acids are present in naturally occurring substances such as insect pheromones and pigments[1,2]. Two trans-2-enoic acids (22:1 and 24:1) have been identified as natural products for the first time[3]. They occur in wheat leaf wax, esterified with C_9-C_{12} α,ω-diols. A number of methods are known to prepare trans-2-enoic acids. LeSueur[4] and others[5,6] prepared one of the two possible geometrical isomers of trans-2-octadecenoic acid along with a by-product, 2-hydroxystearic acid. Palameta and Prostenik[7] obtained trans-2-octadecenoic acid from octadecanoic acid through dehydrohalogenation of 2-bromooctadecanoic acid. Since in this kind of dehydrohalogenation, a trans-elimination mechanism involving a co-planar four-centre transition state[8,9] is operative, there was no cis-unsaturated acid present. Recently it was reported[10] from our laboratory that a co-product, 2-ethoxy-

alkanoic acid was obtained during synthesis of long-chain α,β-unsaturated acids, trans-2-Octadecenoic acid was also prepared[11] by reduction of the 2-acetylenic ester to the cis-alkenoate followed by reaction with mercuric acetate and methanol and then reaction with hydrochloric acid gives (usually) the trans-2-isomer only. The trans-alkenoate can be prepared by stereomutation[12] of the cis-isomers but this requires a tedious separation of cis and trans-isomers.

The α,β-unsaturation in fatty acid is expected to behave differently from the internal olefinic function. If the olefinic bond is very close to the carboxylic function, the behaviour of its isomeric reaction products would be markedly affected by the adjacent carboxylic group through hydrogen bonding. This effect in turn enables them to be separable chromatographically. Ansari and coworkers[13] carried out a systematic study of the hypohalogenation of long-chain α,β-unsaturated acids. The isomeric halohydrins were successfully separated by column chromatography. Similarly, the influence of carboxylic group was markedly observed by them[14] when erythro and threo-2,3-glycols were treated with HBr. However, Husain et al.[15] obtained an isomeric 2(3)-(3'-mercaptopropan-1'-acetoxy-2'-ol) octadecanoic acid from the reaction of trans-2-octadecenoic acid with 3-mercapto-propan-1,2-diol.

Recently a systematic study on allylic halogenation and oxidation of long-chain α,β-unsaturated ester (Ib) was carried out in author's laboratory[16] (Scheme 1). Interestingly besides the normal allylic bromoderivative (IIb), two other products (IV) and (V) were also prepared.

Scheme 1



Recently, Nakayama *et al.*[17] prepared methyl 4-exo-2-alkenoate (VII) by chromium trioxide oxidation.

$$R\text{-}CH_2\text{-}CH{=}CH\text{-}CO_2CH_3 \xrightarrow[Ac_2O\text{-}AcOH]{CrO_3} R\text{-}\underset{\underset{O}{\|}}{C}\text{-}CH{=}CH\text{-}CO_2CH_3$$

15-25 C

(VII)

R=n-C_6H_{13}, C_2H_5-, n-C_3H_7-, n-C_4H_9-, n-C_5H_{11}-, H_3CO_2C-$(CH_2)_7$-

Frighetto and coworkers[18] prepared E,E-2,4-dienoic acid in good yield from α,β-unsaturated ester by allylic halogenation and dehydrohalogenation.

$$CH_3\text{-}(CH_2)_4\text{-}CH_2\text{-}CH{=}CH\text{-}R \xrightarrow{NBS/CCl_4} CH_3\text{-}(CH_2)_4\text{-}\underset{Br}{HC}\text{-}HC{=}CH\text{-}R$$

methyl E-2-nonenoate

R = $-CO_2CH_3$

$$\downarrow \; LiCO_3\text{-}LiCl,\ DMF$$

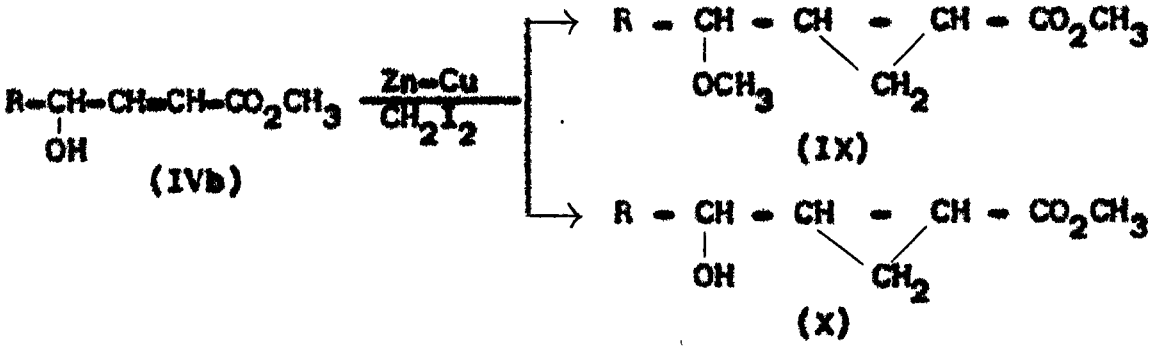
$$CH_3\text{-}(CH_2)_3\text{-}CH{=}CH\text{-}CH{=}CH\text{-}R$$

(VIII)

The stereochemistry of methyl E,E-2,4-nonadienoate (VIII) was assigned on the basis of NMR spectrum and by using lanthanide shift reagent[19].

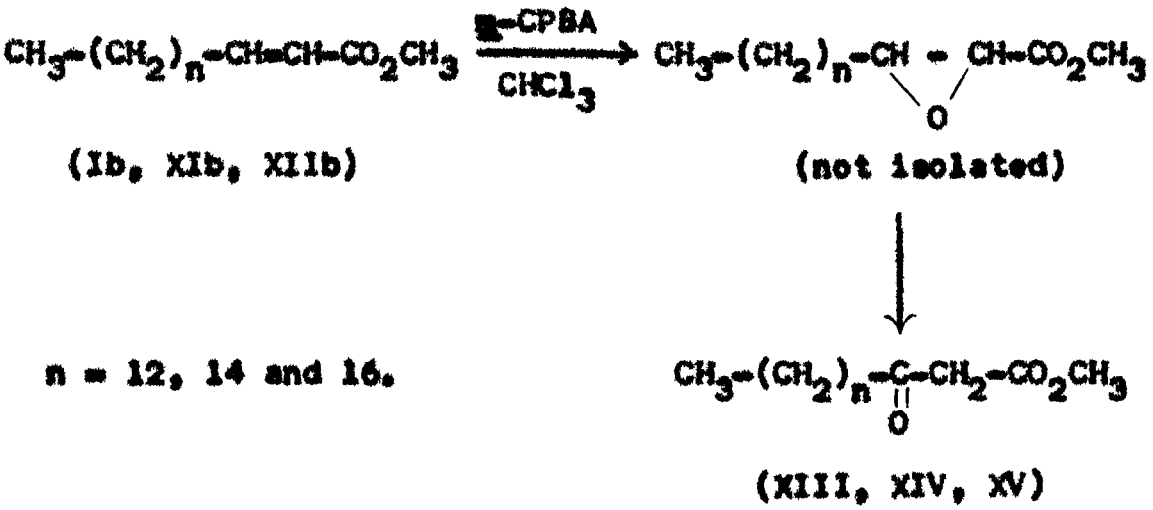
Synthetic cyclepropanes are conveniently prepared by Simmons-Smith reaction (SSR)[20]. In general reaction is completely stereospecific, though a small amount of isomerization may occur in the presence of large excess of zinc-copper

couple[21]. Cyclopropanation was carried out on all the methyl trans-octadecenoates to prepare the corresponding trans-cyclopropanes[22,23]. Recently in our laboratory SSR has been used for the cyclopropanation of methyl 4-hydroxy-trans-2-hexadecenoate (IVb)[24]. It has been noted that the presence of allylic hydroxyl group increases the formation of cyclopropane derivative (yield ~ 90%). The Simmons-Smith cyclopropanation of olefins is very strongly directed by hydroxyl groups[24-27]. The stereospecificity of this reaction has been studied with acyclic allylic alcohols by Ratier et al.[28]

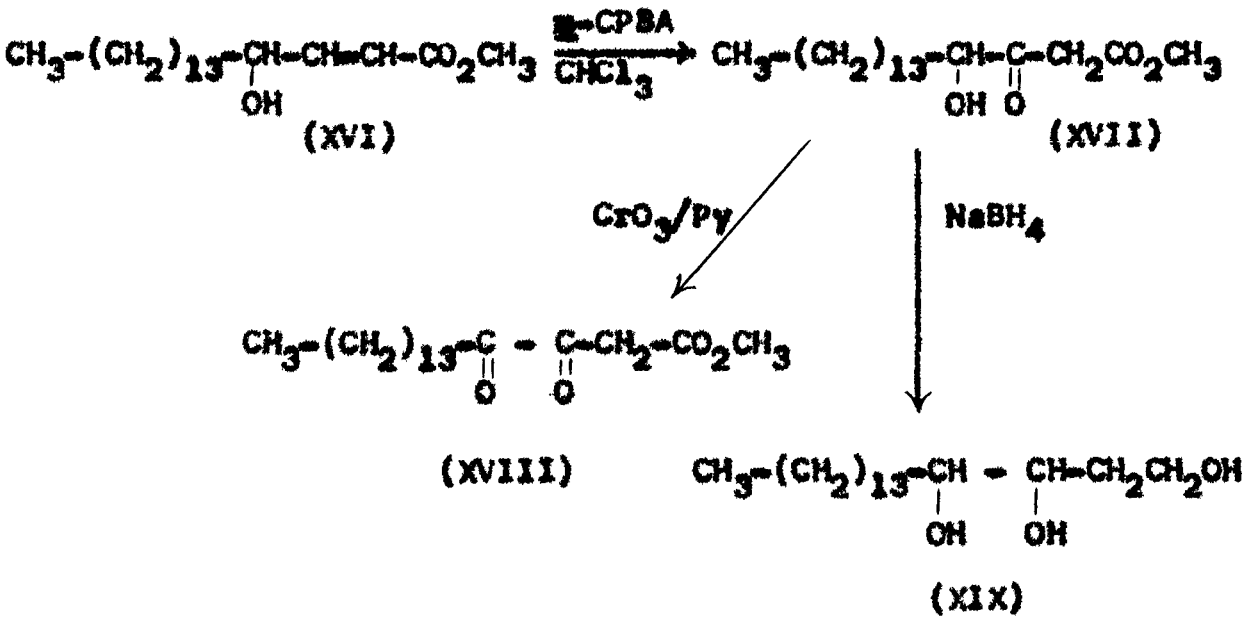


The epoxidation of α,β-olefinic fatty acid or ester with peracids gives only poor yield of epoxide[29]. The discovery of more powerful organic peracids, i.e. m-chloroperbenzoic acid (m-CPBA) and pertrifluoroacetic acid has made it possible to epoxidise less reactive olefinic bonds of α,β-unsaturated compounds. It was reported[30,31] that epoxidation of long-chain α,β-unsaturated acids with perbenzoic and m-CPBA

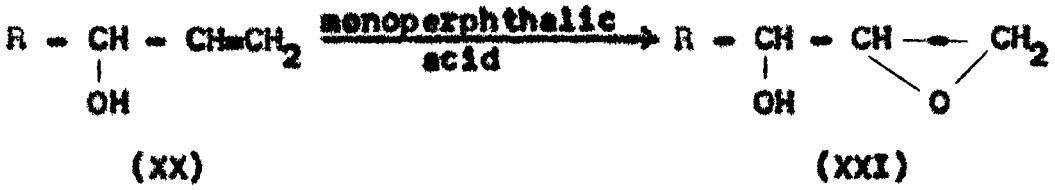
yielded 2,3-epoxy acids. Further studies[32] revealed that the epoxidation of α,β-unsaturated methyl esters with m-CPBA gave a rearranged products, 3-keto esters (XIII-XV) and not the expected 2,3-epoxides. It was suggested that the 2,3-epoxide is unstable and rapidly undergoes intramolecular isomerization to a β-ketoester.



Ansari <u>et al.</u>[33] reported that the oxidation of methyl 4-hydroxy-<u>trans</u>-2-octadecenoate (XVI) with m-CPBA, gave a rearrangement product, methyl 3-keto-4-hydroxyoctadecanoate (XVII). Chromium trioxide-pyridine oxidation and sodium borohydride reduction of XVII yielded methyl 3,4-diketooctadecanoate (XVIII) and 1,3,4-octadecatriol (XIX), respectively.

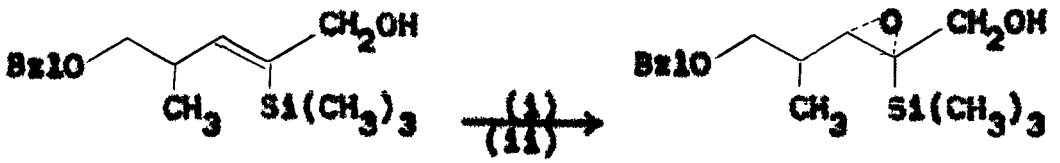


Martin et al.[34] prepared a series of epoxides (XXI) from allylic alcohols (XX).



It was reported[35] that the reaction of olefinic alcohols with m-CPBA in presence of catalylic amount of 2,2,6,6-tetramethyl-piperidine-hydrochloride (TMP-HCl) epoxidises the double bond along with oxidation of alcohol to ketone.

Johnson et al.[36,37] recently reported that epoxidation of allylic alcohols (XXIIa-c) with m-CPBA yielded the epoxides (XXIIIa-c) exclusively in practical sense, while (XXIV) yielded a 3:2 mixture of the two possible epoxides

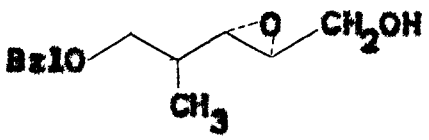


(XXVII) (XXVIII)

(i), m-CPBA

(ii), CH_2Cl_2, OC

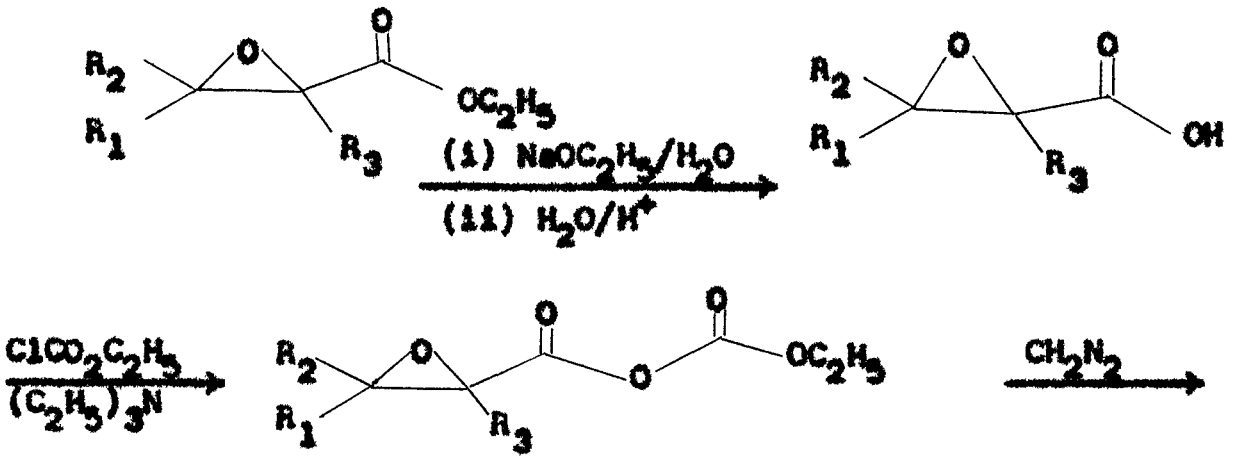
$(n\text{-}Bu)_4NF/DMF$

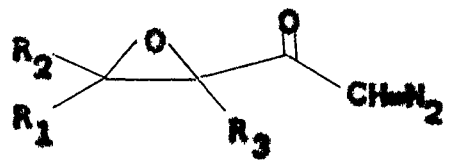


(XXIX)

α,β-Epoxy diazomethyl ketones represent a class of compounds containing two reactive functional groups. These substrates are of interest as they enable to study the chemospecific behaviour of reagents that are reactive towards both epoxides and diazoketones.[39,40]. Recently Thijs et al.[41] reported two general methods for the synthesis of α,β-epoxydiazomethylketone (XXX). Firstly, treatment of mixed anhydrides of glycidic acids and carbonic acid ester with diazomethane led to the α,β-epoxydiazomethyl ketones in yields ranging from 17-74% (Scheme 4). Secondly, glycidyl chlorides which were obtained from sodium glycidates and oxalyl chloride, gave the desired products upon treatment with diazomethane (60-74%) (Scheme 5).

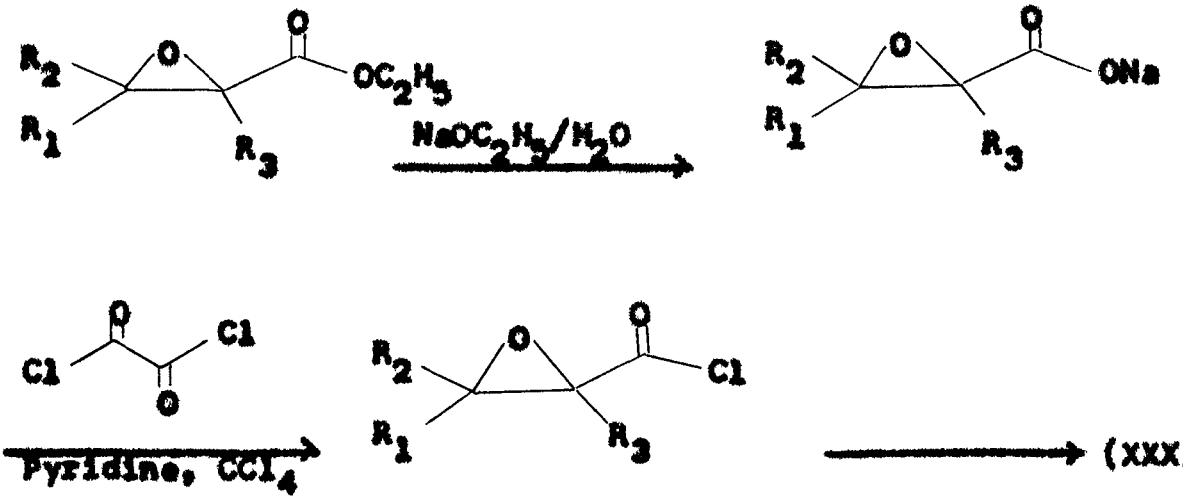
Scheme 4





(XXX)

Scheme 5



Reaction of α,β-epoxydiazomethyl ketones (XXX) with activated copper powder or copper (II) sulphate in methanol (or ethanol) leads to 1,1-dialkyloxy-but-3-ene-2-ones (XXXI) or (XXXII) in good yields[42].

(XXX)

(XXXI) R = CH_3-
(XXXII) R = C_2H_5-

The formation of dimers is reported from the thermal treatment of a series of epoxy fatty acid methyl esters[43].

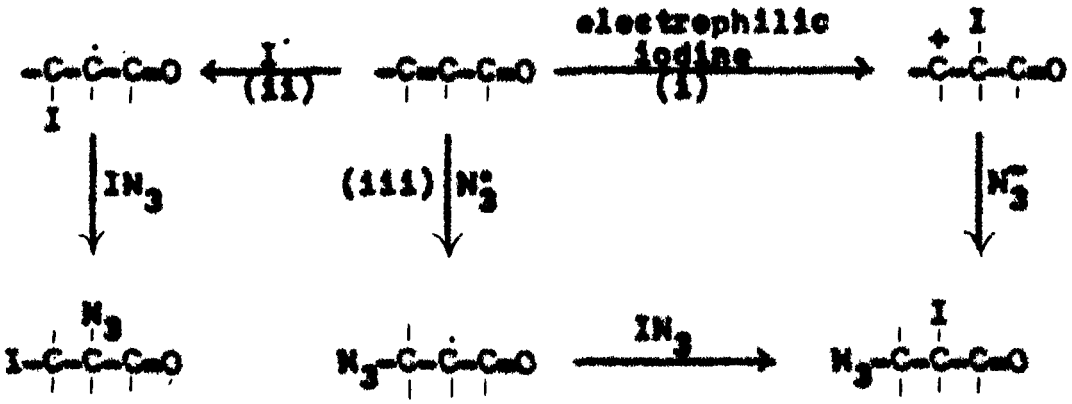
Nitrogen-Containing Derivatives of α,β-Unsaturated Compounds

The fatty acid derivatives, chain-substituted by nitrogen, were with few exceptions rather obscure laboratory curiosity and none have apparently been found from natural sources. In recent years new synthetic methods, especially those involving pseudohalogen addition to olefins, have been perfected and have led to practical procedure by which new nitrogen-substituted fatty acid derivatives can be prepared.

The addition of iodine azide (IN_3) to some α,β-unsaturated esters and ketones has been examined by Hassner

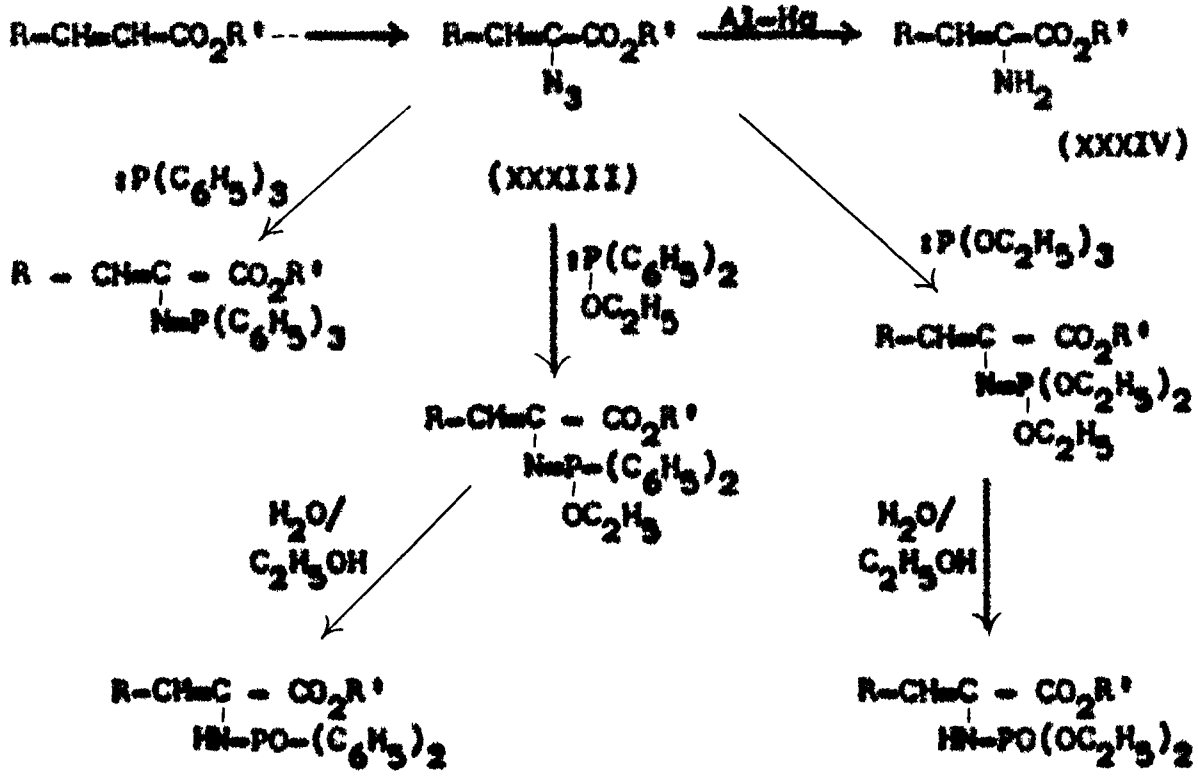
_et al._[44] who proposed a mechanism analogous to that for the addition to alkenes[45] in order to account for the regio- and stereoselectivity of the reactions. More recently Cambie _et al._[46] reported that addition of IN_3 to the α,β-unsaturated esters affords the products consistent with the radical pathway (Scheme 6). Preliminary kinetic results indicate that addition of iodine azide to α,β-unsaturated ketones in the presence of air involves a slow electrophilic attack. The products from addition to the double bond of α,β-unsaturated carbonyl system may be expected to be regiospecific when a species (e.g. electrophilic iodine) which acts as the electrophile in an ionic process (i), behaves also as the radical addend (e.g. an iodine atom) in a competitive homolytic pathway (ii). If, however, the alkyl radical is formed (iii), by delivery of a radical species corresponding to the nucleophilic partner in the ionic process, then the product from either route will be the same.

Scheme 6



Foglia *et al.*[47] investigated the addition of IN_3 to few internal olefins. Addition of IN_3 to methyl *trans*-2-hexadecenoate yielded methyl *erythro*-3-azido-2-iodohexadecenoate[48]. α-Azido-α-alkenoate (XXXIII) and α-amino-α-alkenoate (XXXIV) were prepared by Shin *et al.*[49,50] from α,β-unsaturated acid (Scheme 7). Recently they[51] reported a preparative route for N-protected α-dehydroamino acid with a few kinds of phosphinyl groups by the reaction of ethyl 2-azido-2-alkenoate (XXXIII) with organic tetravalent phosphorous reagent.

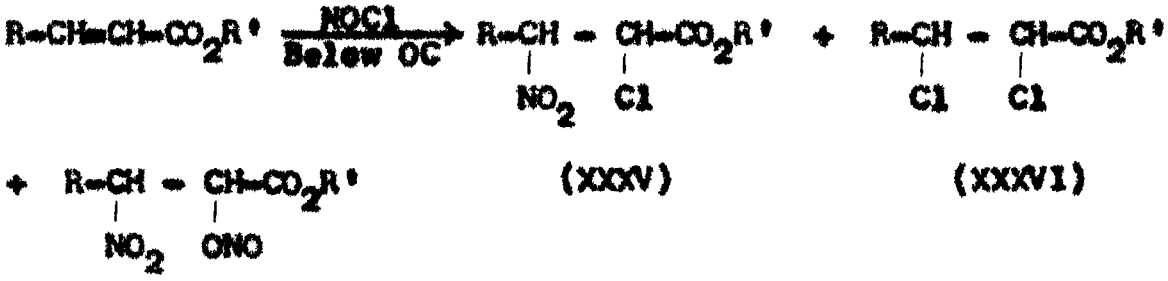
Scheme 7



R = CH_3; C_2H_5; $n\text{-}C_3H_7$; C_6H_5

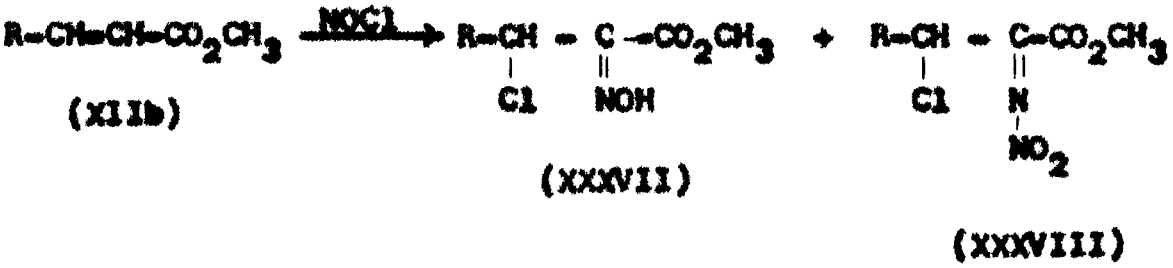
R' = C_2H_5

Further, Shin *et al.*[52] carried out nitrosochlorination on α,β-unsaturated ester and observed that the chief products were XXXV and XXXVI as shown below:



(XXXV) (XXXVI)

When methyl *trans*-2-docosenoate[53] (XIIb) was treated with nitrosochloride (*in situ*) at 0-5C for about a month, only about 10% of compound XIIb was found to undergo nitrosochlorination to give products, methyl 2-eximino-3-chlorodocosanoate (XXXVII) and methyl 2-nitrimino-3-chlorodocosanoate (XXXVIII).

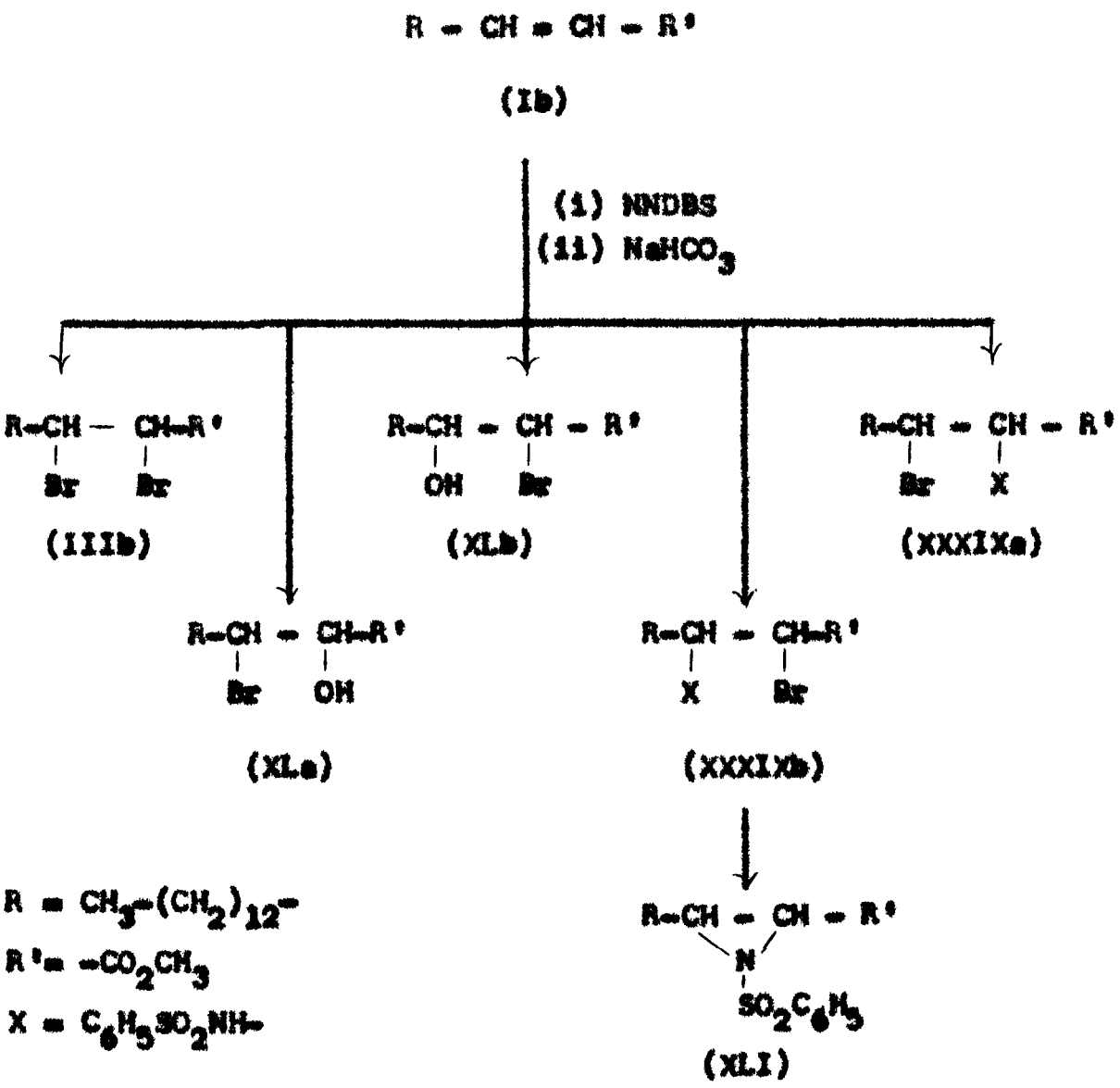


(XIIb) (XXXVII) (XXXVIII)

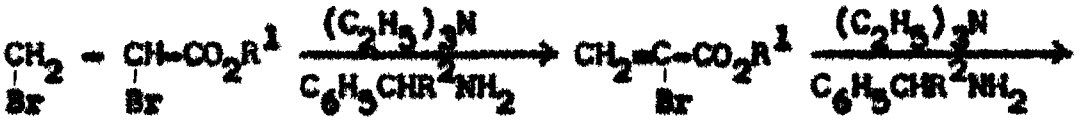
The addition of N,N-dibromobenzene sulphonamide (NNDBS)[54] to methyl *trans*-2-hexadecenoate (Ib, Scheme 8) yielded a mixture of isomeric bromosulphonamde (XXXIXa,b) along with the corresponding dibromide (IIIb) and brome-

hydrins (XLa,b). Chemical cyclization of the major bromosulphenamide (XXXIXb) gave the corresponding aziridine (XLI). The interesting observation was that unlike the internal bromosulphenamide adduct, the isomeric mixture of these derivatives obtained from long-chain α,β-unsaturated ester was successfully resolved by column chromatography.

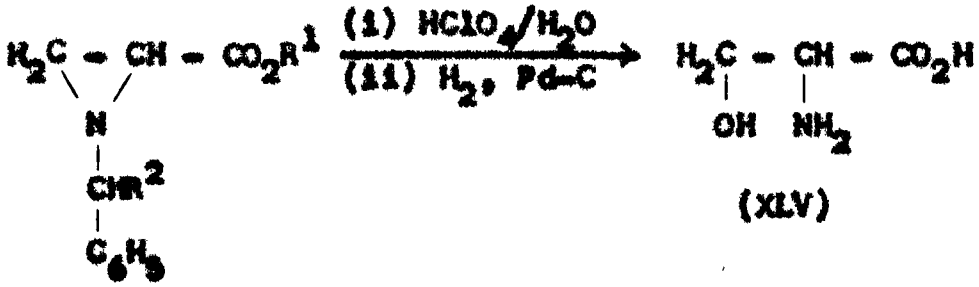
Scheme 8



Synthetic and stereochemical studies of aziridine 2-carboxylic acids derived from short-chain α-bromo-α,β-unsaturated or α,β-dibromocarboxylic acid esters have been reported[53-59]. Harada *et al.*[60] described the asymmetric synthesis of the alkyl aziridine carboxylate (XLIV) from α,β-dibromopropionate (XLII) and chiral benzyl amines and the formation of optically active serine (XLV) by hydration of the aziridine.



(XLII) (XLIII)



(XLIV) (XLV)

R^1 = CH_3, C_2H_5, i-Pr, or t-Bu

R^2 = CH_3 or C_2H_5

The foregoing account of the reactions of α,β-unsaturated fatty compounds clearly points out that compared

to the chemistry of internal olefinic fatty substrates, α,β-unsaturation has attracted less attention of fat chemists. During recent years a number of novel reactions using new reagents have been carried out preferably with short-chain and cyclic substrates containing unsaturation. But very little work elsewhere has been published on the reactions of long-chain α,β-unsaturated fatty compounds. These considerations led to the present series of investigations as a continuing project of research carried out in the author's laboratory on long-chain olefinic fatty substrates and their oxygenated derivatives.

The discussion and experimental part of the thesis give an account of the series of reactions carried out in the present study.

Discussion

3. Peracid Oxidation of α,β-Unsaturated Fatty Acids and their Derivatives

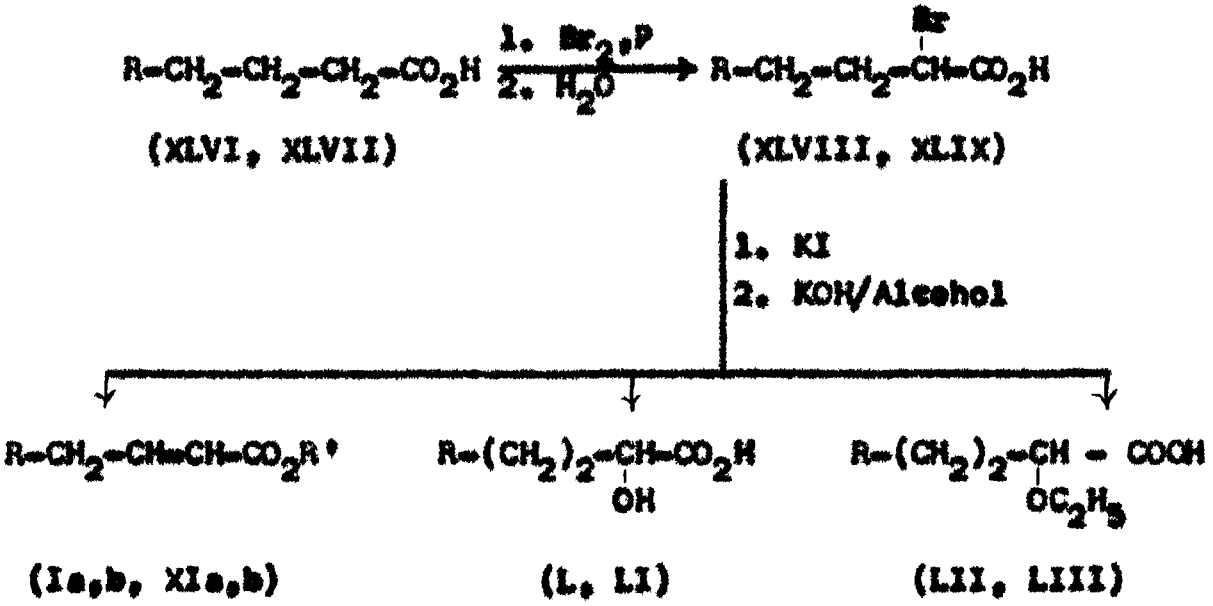
Recent studies of long-chain hydroxy alkenes containing the unit $-CH=CH-(CH_2)_n-\underset{OH}{CH}-$ have shown that neighbouring group participation may occur between the hydroxyl group and the double bond depending particularly on the value of n and to some extent on the geometry of the double bond. The peracid oxidation (epoxidation) of ricinoleic, a β-hydroxy-olefinic acid gives the hydroxy epoxide while isoricinoleic (γ-hydroxyolefinic) acid furnishes 9,12-epoxide[61].

Long-chain fatty compounds with an allylic substituent ($-\underset{X}{CH}-CH=CH-$) are rarely found in natural fats. Compounds containing the above moiety are expected to possess greater reactivity than the simple olefinic compounds. More so, when the allylic substituent occupies a position nearest the carboxylic end of the fatty acid chain. Sporadic reports have appeared in the literature on the reactions of fatty compounds containing the above allylic grouping. Keeping in view the scanty literature on the chemistry of such unusual compounds, the present work on the epoxidation of such substrates, viz: methyl trans-2-enoates (Ib, XIb), methyl 4-hydroxy-trans-2-hexadecenoate (IVb), trans-2-octadecen-1-ol (LIV) and methyl 4-oxo-trans-2-enoates (VI, LV) has been undertaken to synthesize new fatty acid derivatives. The preparation of above substrates and their epoxidation results are given and discussed under separate headings.

3.1A. Preparation of trans-2-Enoic Acids (Ia, XIa)

trans-2-Enoic acids of C_{16} and C_{18} chain length were prepared[7,10] from palmitic (XLVI) and stearic (XLVII) acids, respectively (Scheme 9). The structure of trans-2-enoic acids

Scheme 9



XLVI, XLVIII, Ia,b, L, LII; R = $CH_3-(CH_2)_{11}-$

XLVII, XLIX, XIa,b, LI, LIII; R = $CH_3-(CH_2)_{13}-$

Ia,XIa; R' = H

Ib,XIb; R' = CH_3-

(Ia, XIa) were established by their elemental analyses and the spectral study of their methyl esters (Ib, XIb). The compounds Ib and XIb had the characteristic IR absorption bands at 1730

(-CH=CH-COOCH$_3$), 1650 (-CH=CH-) and 980 cm^{-1} (trans-double bond). The NMR spectra gave doublet of doublet centered at τ 3.1 (1H, J=15 and 5 Hz) ascribable to a proton β to ester carbonyl, a doublet at τ 4.0 (1H, J=15 Hz with a small long range coupling, trans-olefinic proton) attributed to a proton α to ester carbonyl, 7.98 m (2H, -CH$_2$-CH=CH-) and usual signals as observed in fatty acid esters. The coupling constant established that the configuration of double bond is trans.

3.18. Preparation of Methyl-4-hydroxy-trans-2-hexadecenoate (IVb)

Methyl-4-hydroxy-trans-2-hexadecenoate (IVb, m.p.56-57C) was prepared[16] from methyl trans-2-hexadecenoate (Ib). Compound Ib on reaction with NBS (2 mole) afforded allylically brominated ester (80%) which on alkaline hydrolysis yielded the corresponding allylic hydroxy acid (IV, 70%, m.p. 70-71) (Scheme 1).

The structure of 4-hydroxy-trans-2-hexadecenoic acid (IVa) was confirmed by microanalysis and spectral data of its methyl ester (IVb). Compound IVb analyzed for $C_{17}H_{32}O_3$. The IR spectrum gave bands at 3350-3270 (OH), 1730 (-COOCH$_3$), 1660 (-HC=CH-), 1170, 1140, 1080, 1050 (C-O) and 980 cm^{-1} (trans-unsaturation). The NMR spectrum gave characteristic signals at τ 3.1 d,d (1H, -CH$_C$-CH$_B$=CH$_A$-CO$_2$CH$_3$, J_{AB}=15 Hz and J_{BC}=5 Hz),

4.0 d (1H, $-CH=\underline{CH}-CO_2CH_3$, J=15 Hz with a small long range coupling), 5.8 br (1H, $-\underline{CH}-OH$) and 7.6 br (1H, $O\underline{H}$, D_2O exchangeable).

3.1C. Preparation of trans-2-Octadecen-1-ol (LIV)

The selective reduction[62] of methyl trans-2-octadecenoate (XIb) with lithium aluminium hydride (LAH) gave trans-2-octadecen-1-ol (LIV) (Scheme 10).

Scheme 10

$$CH_3-(CH_2)_{14}-CH=CH-CO_2CH_3 \xrightarrow[Ether]{LAH} CH_3-(CH_2)_{14}-CH=CH-CH_2OH$$

(XIb) (LIV)

Compound LIV (m.p.46-47C) (lit. m.p.45-48)[63] exhibited the elemental composition $C_{18}H_{36}O$ as supported by microanalysis. Its IR spectrum furnished bands at 3280 cm^{-1} for hydroxyl group and 950 cm^{-1} for trans-unsaturation. The band at 1730 cm^{-1} ($\underline{CO}OCH_3$) is absent in the IR spectrum of compound LIV. NMR spectrum gave signals at τ 4.7 m (2H, $-CH_2-\underline{CH}=\underline{CH}-CH_2OH$), 6.43, unresolved doublet (2H, $-\underline{CH_2}-OH$), 6.54 br,s (1H, $-CH_2\underline{OH}$) and 8.04 m (2H, $-\underline{CH_2}-CH=CH-$). After deuterium exchange the signal at τ 6.54 disappeared with a small change in the signal at τ 6.43. Thus on the basis of above data the structure of compound LIV was assigned as trans-2-octadecen-1-ol.

3.10. Preparation of Methyl 4-oxo-*trans*-2-enoates (VI and LV)

The allylic oxidation of methyl *trans*-2-hexa- (Ib) and octadecenoate (XIb) with chromium trioxide[17] in acetic anhydride and acetic acid yielded methyl 4-oxo-*trans*-2-hexadecenoate (VI) (~ 84%, m.p.64-65C) (lit. m.p.65-66C)[16] and methyl 4-oxo-*trans*-2-octadecenoate (LV) (~ 83.5%, m.p.68C), respectively (Scheme 11).

Scheme 11

$$\underset{\text{(Ib, XIb)}}{R\text{-}CH_2\text{-}CH{=}CH\text{-}CO_2CH_3} \xrightarrow[Ac_2O\text{-}AcOH]{CrO_3} \underset{\text{(VI, LV)}}{R\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH{=}CH\text{-}CO_2CH_3}$$

Analytical values of compounds VI and LV corresponded to molecular formula $C_{17}H_{30}O_3$ and $C_{19}H_{34}O_3$, respectively and gave positive DNP test. UV spectra (λmax. 220 nm) show these to have S-*trans*, S-*trans*-configuration. The IR (KBr) spectra exhibited the bands at 1730 (-HC=CH-<u>C</u>OOCH$_3$), 1665 (-<u>C</u>OCH=CH-), 1640, very intense (vinyl-unsaturation), 1270, 1210, 1190 (C-O) and 995 (*trans*-unsaturation). Elemental composition, IR and UV suggest that a conjugated di-oxoalkenoate was formed. The NMR signals which unequivocally established the conjugated di-oxoalkenoate structure was a doublet at τ 2.95 (J=16 Hz) integrated for one proton. This signal is attributed

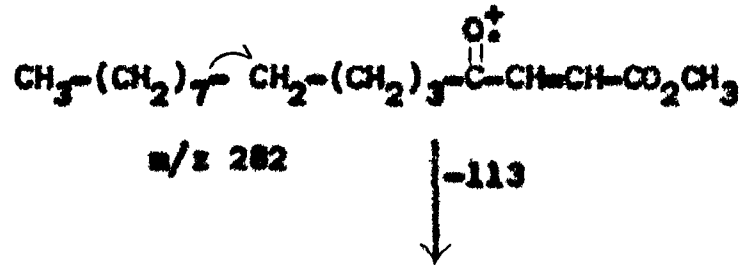
to the vinylic proton β to ester carbonyl. A doublet of same magnitude for one proton was observed at τ 3.46 (J=16 Hz, trans-olefinic proton), ascribable to proton α to ester carbonyl. The signal integrating for two protons is due to the α-methylene proton of C_5, appeared at τ 7.5 as a multiplet.

The mass spectrum of VI (Fig. 1) gave molecular ion (M^+) peak at m/z 282 and other prominent peaks at m/z 251 (M-31), 250 (M-32), 223 (M-59), 169 (δ-cleavage between C_8-C_9), 155 (γ-cleavage between C_7-C_8 or 169-14), 141 (β-cleavage between C_6-C_7), 137 (169-32), 128 (McLafferty cleavage, base peak), 123 (155-32), 113 (128-15 or cleavage between C_4-C_5), 109 (137-28), 97 (128-31), 96 (128-32), 95.

In the absence of accurate mass spectrum genesis of some significant ions are explained and may be treated as tentative.

m/z 169 (M-113) and 155 (169-14)

Fragment ion m/z 169 arises from molecular ion by the loss of mass unit 113. This fragment on further loss of 14 mass unit gives rise to a fragment ion at m/z 155.



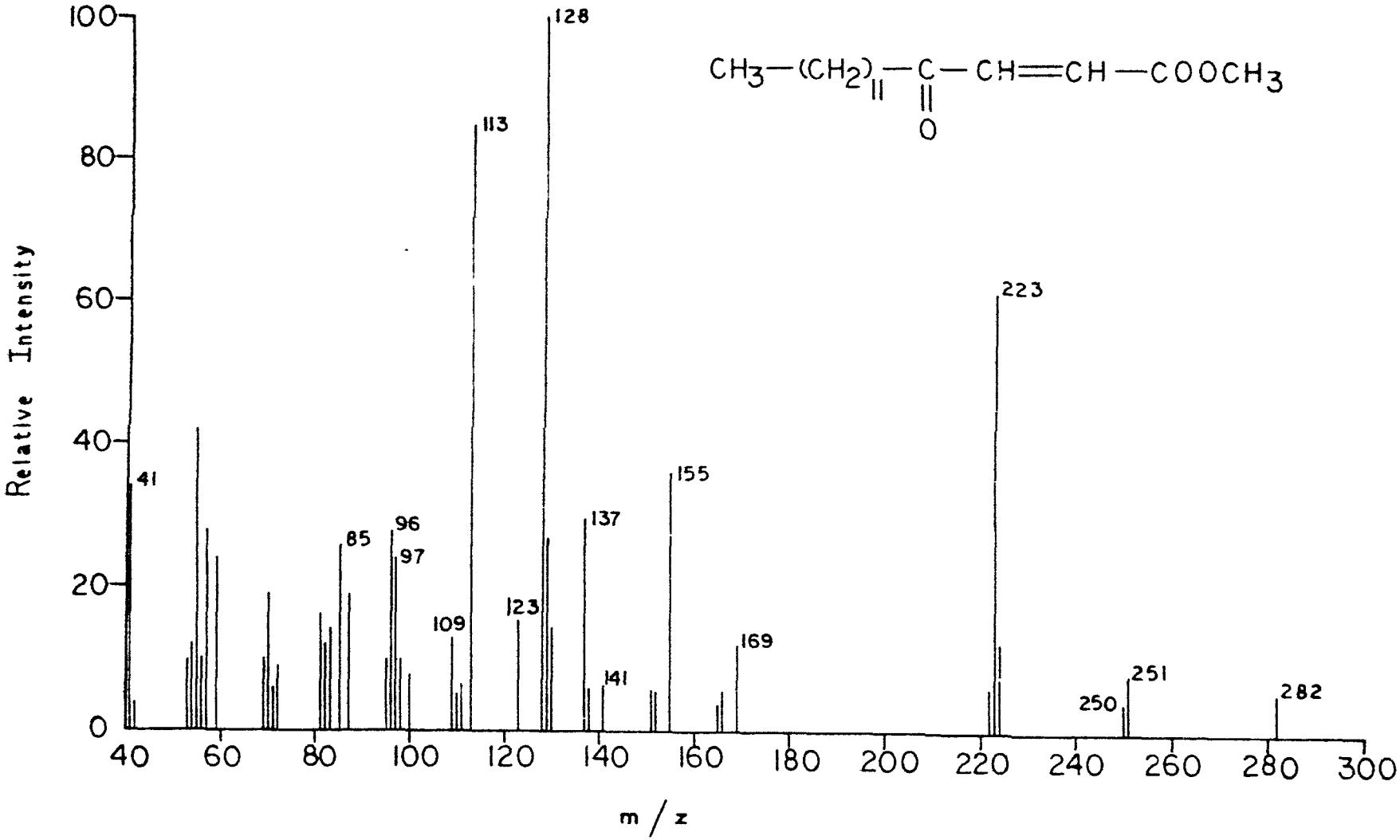
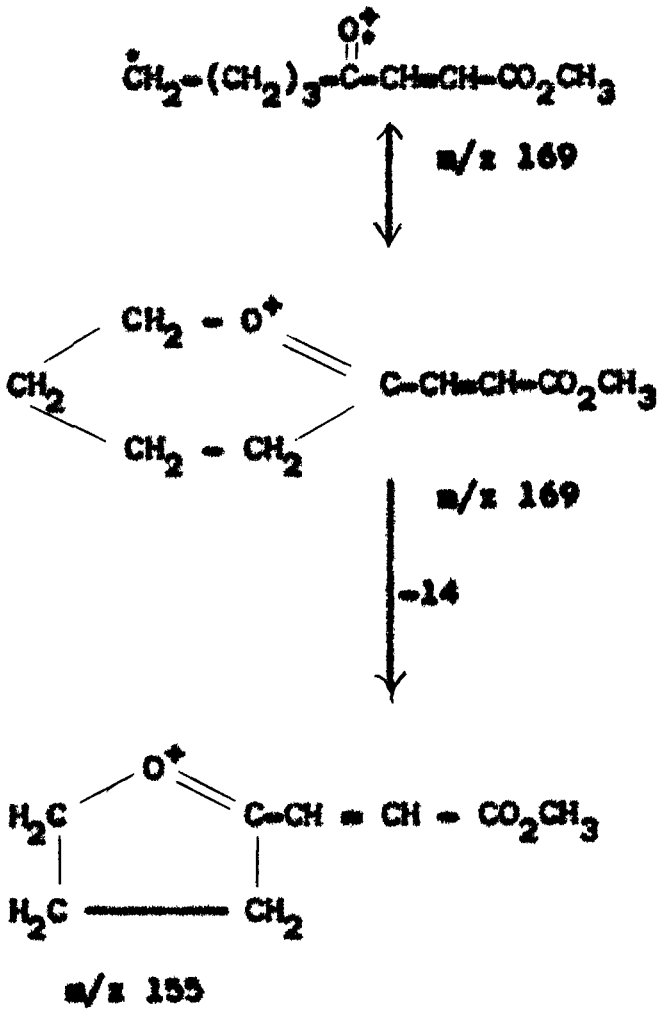
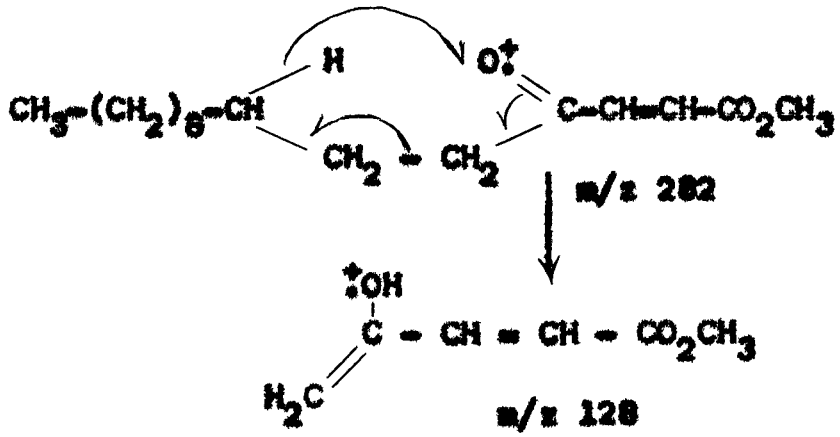

100
80
60
40
20
0
Relative Intensity
128
113
223
155
137
96
85
97
41
123
109
169
141
251
250
282
40
60
80
100
120
140
160
180
200
220
240
260
280
300
m / z
CH3—(CH2)—C—CH=CH—COOCH3
O

FIG. 1. MS of VI



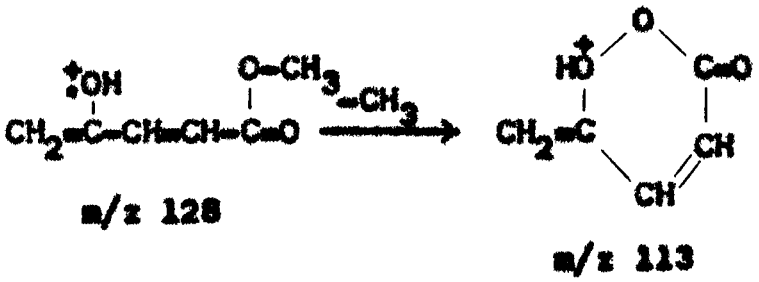
m/z 128

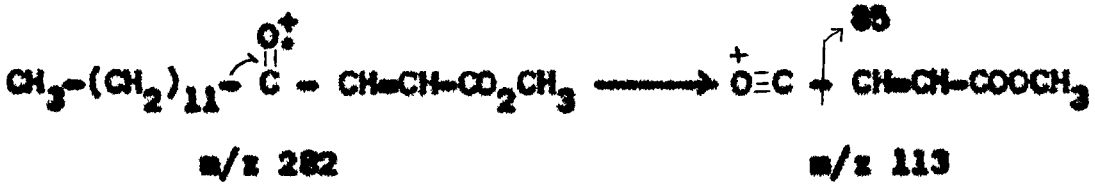
The fragment ion m/z 128 results by the loss of mass unit 154 from the molecular ion through the normal McLafferty rearrangement and thus confirms the position of oxo group at C_4.



m/z 113 (128-15)

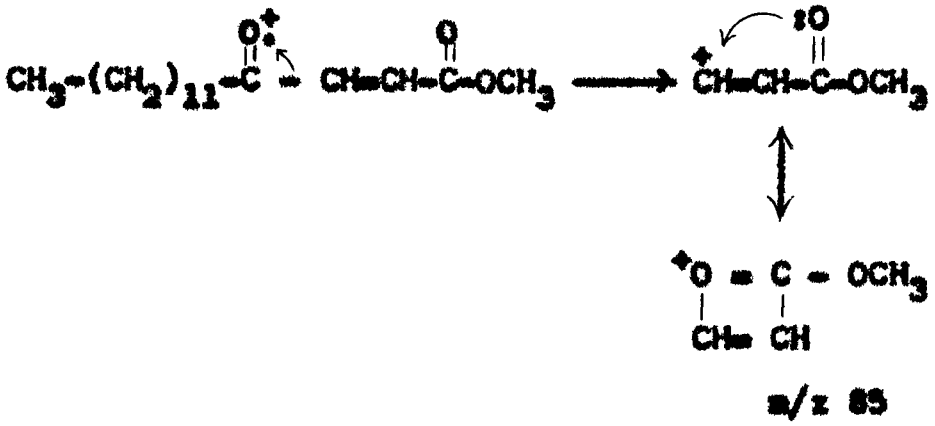
This fragment ion can be shown to arise by the loss of mass unit 15 from the fragment ion 128 or from molecular ion as shown below;





m/z 85 (m/z 113-CO or M-197)

The following mechanism has been proposed to account for the loss of 197 from the molecular ion.



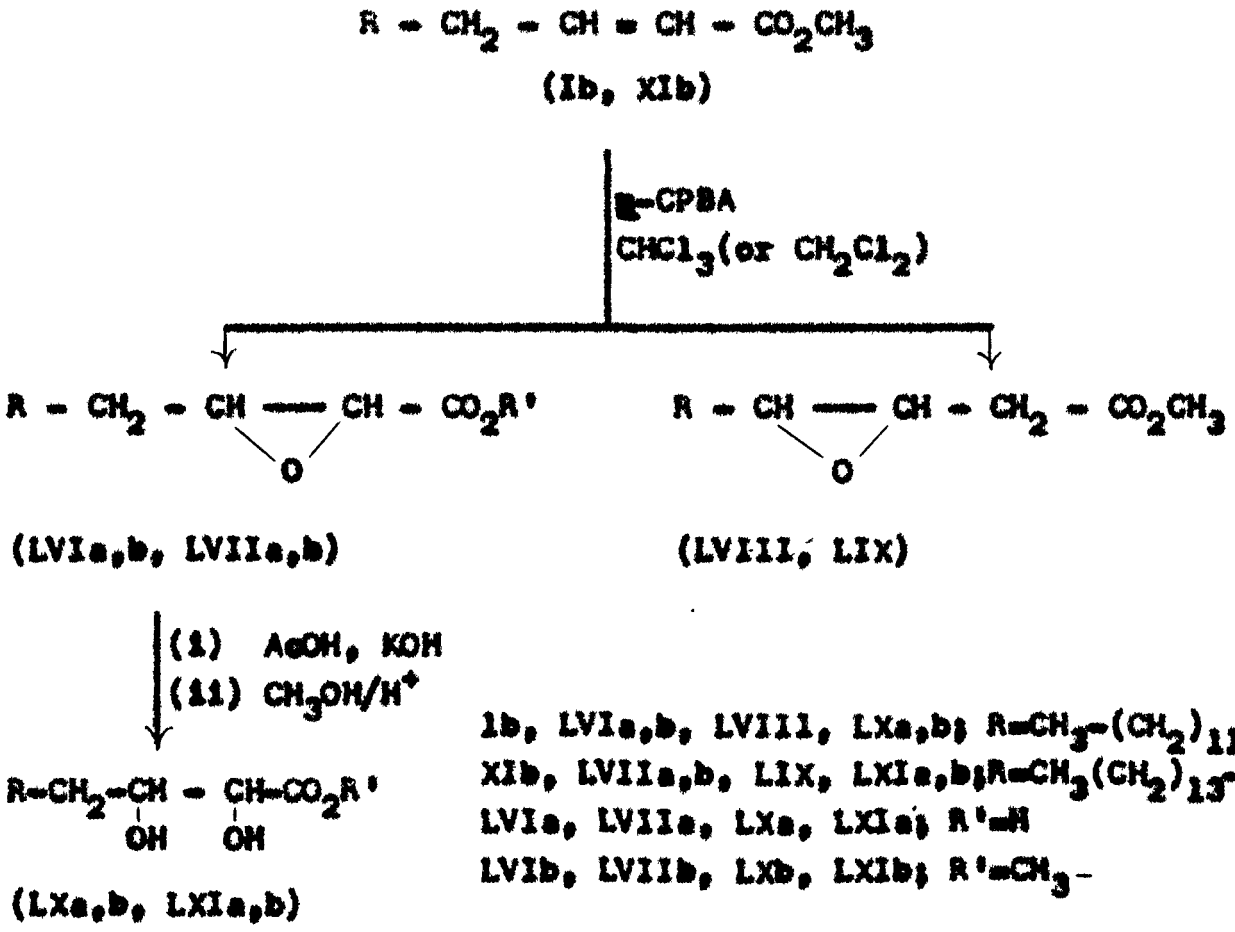
Preparation of methyl 4-oxo-trans-2-alkenoates (VI and LV) from α,β-unsaturated esters by chromium trioxide oxidation in a mixture of acetic anhydride and acetic acid is a simple, rapid, efficient and time saving procedure as compared to the method described by Ahmad et al.[16] Such compounds are also of interest in fat chemistry and can be converted to cyclopentenones[64].

3.2A. Peracid Oxidation of Methyl trans-2-hexa-(Ib) and -octadecenoate (XIb)

Reaction of methyl trans-2-enoates (Ib) and (XIb) was carried out in chloroform following the procedure of Gunstone and Jacobsberg[31] using m-CPBA as an oxidant. The reaction mixture was kept at room temperature (10 days) and the progress of reaction was monitored by TLC. Direct TLC of the reaction mixture showed three spots corresponding to unreacted methyl esters (Ib, XIb), a dense one (LVIb and LVIIb, ~ 65%) and a faint

spot (LVIII and LIX; ~ 3.8%) for epoxides (Scheme 12). These results differed with the previous reports on epoxidation of methyl trans-2-enoates where either 2,3-epoxides[30,31] or 3-keto esters[32] was the sole product. Epoxidation of Ib and XIb with a change in reaction condition (58 hr., refluxing in methylene dichloride) only affected the yield of epoxides (LVIb and LVIIb in 70%) and (LVIII and LIX in 4.3%). The individual products were separated with the help of silica gel column.

Scheme 12



Characterization of Products (LVIb and LVIIb)

The compound LVIb (m.p.35-36C) and LVIIb (m.p.42-43C) analyzed for $C_{17}H_{32}O_3$ and $C_{19}H_{36}O_3$, respectively. Evidences for their structure were obtained from IR, NMR, ^{13}C NMR and MS data as well as from chemical methods.

The IR spectra displayed a strong absorption at 890 cm^{-1}, characteristic for trans-epoxy group. Two partially resolved absorption bands, 1750 and 1735 cm^{-1} were observed which are attributable to the stretching vibration of carbonyl function. When the IR spectra of these compounds were examined in chloroform, it was apparent that two bands at 1745 and 1725 were still present, the second band being a shoulder. This effect has been ascribed to the existence of two conformational isomers (A) and (B)[65]. A normal ester band is expected from conformational isomer (B) since the C=O and C-O dipoles are oriented in opposite directions. The second band, relatively

(A) (B)

of higher frequency (i.e. increased carbonyl character) than the first (B) is associated with conformation (A) having parallel C=O and C-O dipoles. The absorptions at 1205 and 1240 cm^{-1} are as a result of the ring breathing vibration

(C=C and C=O bonds all stretching in phase)[66] and 1190 and 1175 cm^{-1} (C-O). The CH group in the ring absorption was observed at 3000 cm^{-1}. The NMR spectra exhibited signals a broad one centered at τ 6.88 for two protons ascribable for oxirane protons, 6.24 s (3H, $-CO_2CH_3$), 8.7 br,s (chain $-CH_2-$) and 9.12 t (3H, CH_3-). On the basis of these data compounds LVIb and LVIIb were characterized as methyl trans-2,3-epoxy-hexadecanoate and methyl trans-2,3-epoxyoctadecanoate, respectively.

Further support for structure LVIb was obtained from ^{13}C NMR and MS data. ^{13}C NMR chemical shifts (ppm) are given in table 1.

Table 1

^{13}C NMR chemical shifts of compound LVIb and LVIII.

	LVIb	LVIII	LVIb		LVIII
OCH_3	52.92	52.49	C_6	29.68	26.43
C_1	169.72	171.02	C_7-C_{12}	29.52	29.47
C_2	32.22	33.74	C_{13}	29.36	29.36
C_3	58.45	51.84	C_{14}	31.96	31.96
C_4	31.47	56.66	C_{15}	22.69	22.69
C_5	25.73	29.63	C_{16}	14.08	14.08

The MS of LVIb and LVIIb gave molecular ion peaks at m/z 284 and 312, respectively. Besides the molecular ion, other significant peaks observed in LVIb (Fig.2) were at m/z 266 (M-18), 253 (M-31), 252 (M-32), 227 (M-57), 225 (M-59), 224 (M-60), 213, 211 (M-73), 210 (211-H), 206 (224-18), 192 (210-18), 181 (210-CHO), 182 (252-70), 168 (182-14), 166, 164 (182-18), 154 (168-14), 148 (166-18), 140 (154-14), 128, 122 (140-18), 116, 115 (M-169), 112 (140-28), 110 (128-18), 100 (128-28), 96, 94 (112-18), 89 (M-195), 82 (96-14), 80 (94-14), 73 (89-16), 70 (112-42), 68 (96-28 or 100-32), 66, 56 (115-59), 54 (base peak). The formation of some important structure-revealing fragment ions has been proposed to occur according to schemes given below (Chart 1).

<u>m/z 225 (M-59)</u>

The formation of this fragment ion m/z 225 results by the loss of $-CO_2CH_3$ group from the molecular ion. Normally, a methyl ester does not lose mass unit 59 in simple cases, though theoretically possible. This view finds support from the mass spectral data of several fatty methyl esters[67]. The resultant positively charged species is not stabilized by relevant factors.

$$R\text{-}CH_2\text{-}CO_2CH_3 \xrightarrow{-OCH_3} R\text{-}CH_2\text{-}C\equiv O^+ \xrightarrow{-CO} R\text{-}\overset{+}{C}H_2$$

(not favoured)

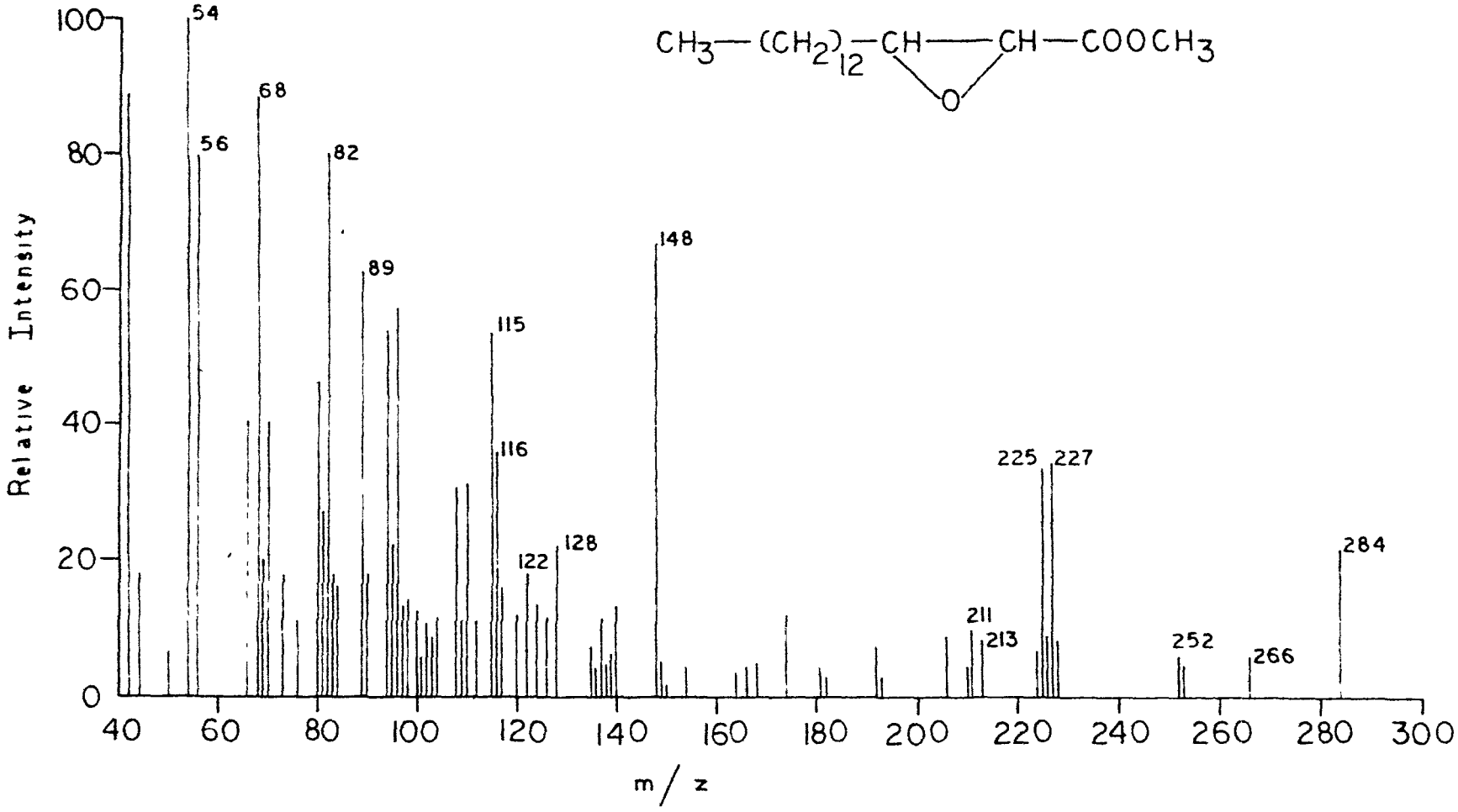
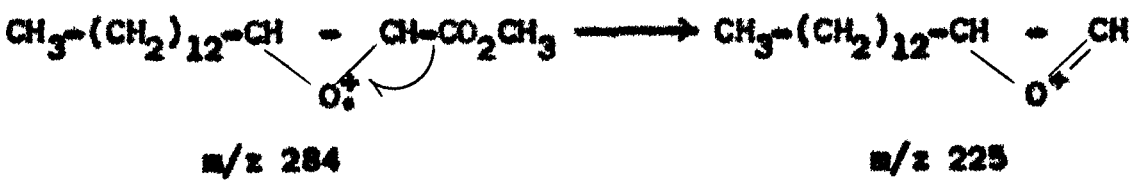


FIG.2 M S of LVIb

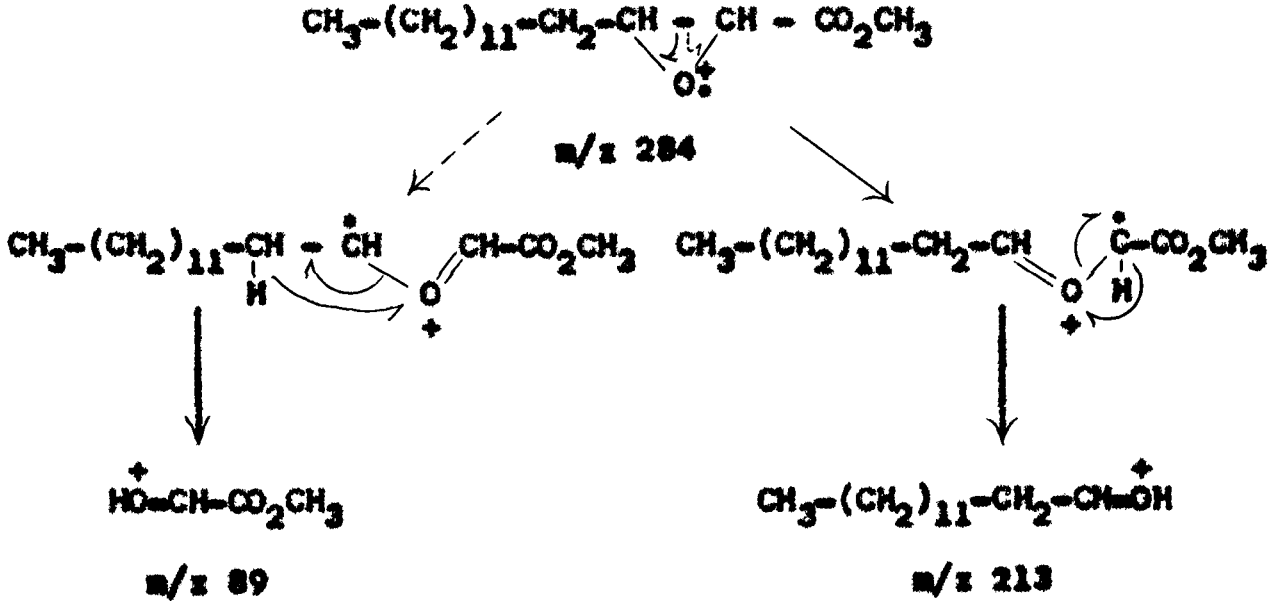
On the other hand if a heteroatom substituent is present at C_2, then the loss of $-CO_2CH_3$ from the molecular ion is favourable as observed in this case.



m/z 284 m/z 225

m/z 213 (M-71) and 89 (M-195)

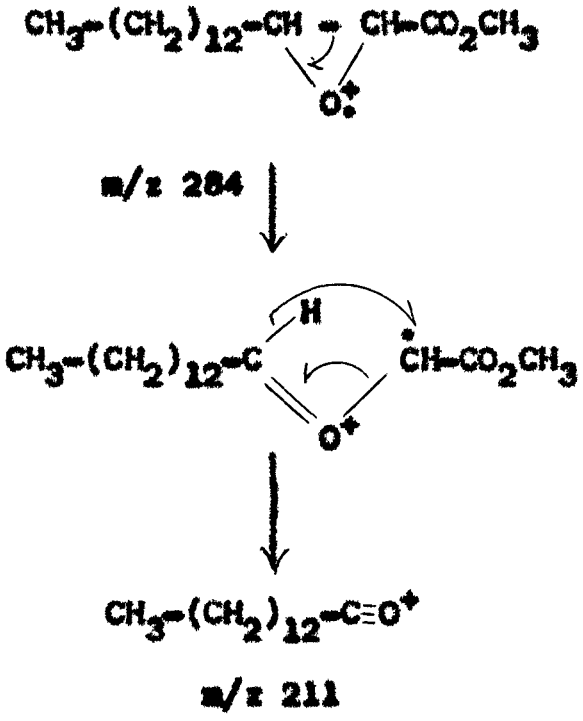
The prominent ions m/z 213 and 89 obviously result by the transannular fragmentation with concomitant hydrogen transfer from the molecular ion. This is a characteristic fragmentation pattern of epoxy compounds and confirms the position of epoxy group at C_2-C_3.



m/z 89 m/z 213

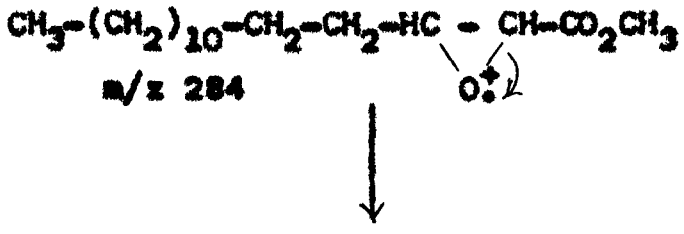
m/z 211 (M-73)

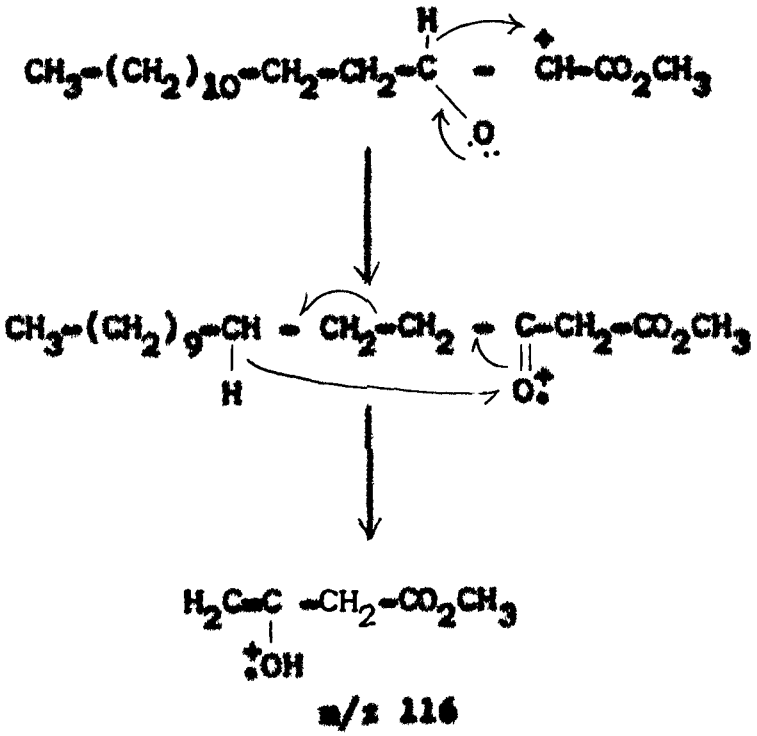
The fragment ion m/z 211 arises from the molecular ion by the loss of mass unit 73, via ring fission.



m/z 116 (M-168)

This fragment ion of higher intensity can be shown to arise by ring opening followed by McLafferty rearrangement.





The pure epoxides (LVIb, LVIIb) when subjected to acetolysis followed by saponification, gave the corresponding erythro-2,3-dihydroxyhexadecanoic (LXa) m.p.102C (lit. m.p. 103-104)[14] and erythro-2,3-dihydroxyoctadecanoic acid (LXIa) m.p.105C (lit. m.p.106C)[7]. The boric acid TLC of LXb and LXIb showed the identical behaviour with the authentic samples of methyl erythro-2,3-dihydroxyhexa- and octadecanoate. Products LXb and LXIb were analyzed for $C_{17}H_{34}O_4$ and $C_{19}H_{38}O_4$, respectively. IR spectra gave bands at 3400 (OH), 1730 ($\underline{CO}OCH_3$) and 1120, 1070 cm^{-1} (C-O).

The compounds LVIb and LVIIb on refluxing with methanolic potassium hydroxide yielded their corresponding

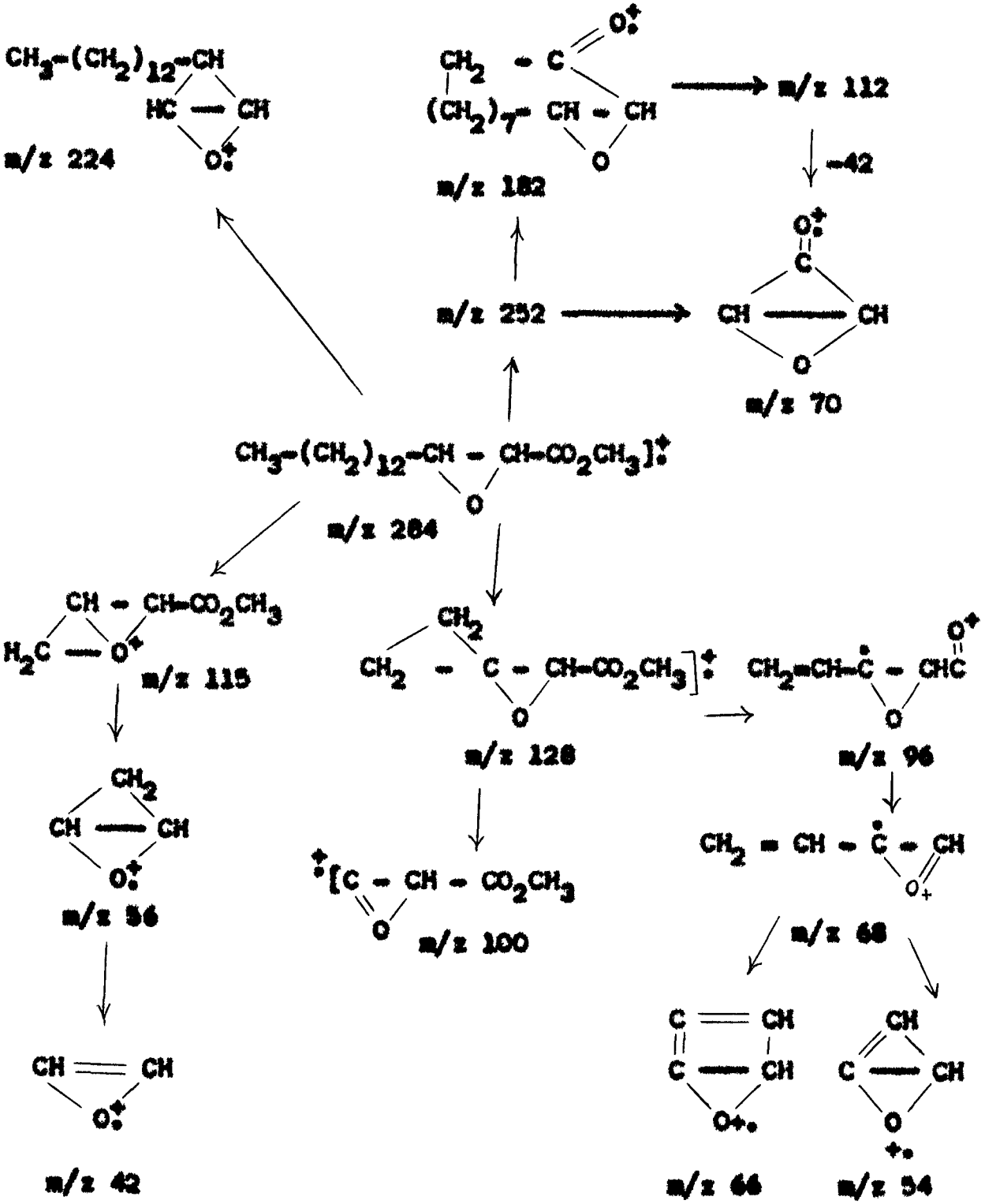


Chart 1. Mass Fragmentation of LVIb

acids, LVIa (m.p. 81C) and LVIIa (m.p.86C) (lit. m.p.85-86C)[31]. Their IR spectra gave broad band 3380- 3160 (COOH), two bands of almost equal intensity in the carbonyl region i.e. at 1730 and 1705 cm^{-1} (COOH), 1070 (C-O) and 890 cm^{-1} (trans-epoxy ring).

Characterization of Minor Products (LVIII and LIX)

Elemental analyses corresponded to molecular formulae $C_{17}H_{32}O_2$ and $C_{19}H_{36}O_3$ for compounds LVIII (m.p.44-45C) and LIX (m.p.49.5C). The IR spectra of these products exhibited the bands at 3010 and 2990 cm^{-1} for CH and CH_2 stretchings, respectively and a band at 1730 cm^{-1} for ester carbonyl. The intense band at 840 cm^{-1} was ascribable to cis-epoxy group. The other bands observed were 1265, 1225, 1190, 1165 and 1030 (C-O). The NMR spectra gave conclusive support in favour of structures LVIII and LIX. Besides the usual NMR signals of fatty compounds other signals observed were at τ 6.27 s (3H, $-CO_2CH_3$), 6.73 m (1H, $-CH-CH-CH_2CO_2CH_3$) and 7.04 m (1H, $-CH-CH-CH_2CO_2CH_3$). The observed values for epoxide protons are slightly different from those previously reported[31] (τ 6.8 and 7.15 for cis-3,4-epoxide). The doublet characteristic of sandwitched CH_2 was centered at τ 7.46 (J=2 Hz) integrated for two protons. On the basis of above mentioned data the assigned structures of compounds LVIII and LIX were methyl cis-3,4-epoxyhexa- and

octadecanoate respectively. Additional evidence for LVIII comes from the ^{13}C NMR spectrum (Table 1).

The mass spectrum of compound LVIII (Fig.3) gave the molecular ion peak at m/z 284 ($C_{17}H_{32}O_3$) along with other salient ion peaks at m/z 266 (M-18), 253 (M-31), 252 (M-32), 241 (M-43), 235 (266-31), 211 (M-73), 199 (M-85), 197 (M-87), 193 (211-18), 192 (193-H), 185 (M-99), 187, 181 (241-60), 180 (241-61), 167 (181-14), 157, 154 (167-13), 151, 149 (167-18), 143, 129, 126 (157-31), 125 (157-32), 115 (M-169), 112 (143-31), 111 (143-32), 103 (M-181), 101 (M-183), 98 (157-59 or 129-31), 97 (111-14 or 129-32), 87 (M-197 or 115-28), 84 (143-59 or 115-31), 83, 71 and 70. The genesis of some important fragment ions are outlined in chart 2.

Fragment ions m/z 211 and 115 originated by the α-cleavages and thus locate the position of epoxy group at C_3-C_4. Fragment ion m/z 211 is much more abundant than the ion m/z 115. The ions m/z 199, 197, 103, 101 and 87 also confirm the position of epoxy group and are the rearranged ions, characteristic of epoxy function. The β, γ and δ-cleavages were observed at m/z 129, 143 and 157, respectively.

The stable α,β-unsaturation in presence of acid[68] (p-CBA) remains in equilibrium to some extent with β,γ-unsaturation in solution. Consequently the latter leads to the formation of β,γ-epoxide (3,4-epoxide) as a minor product.

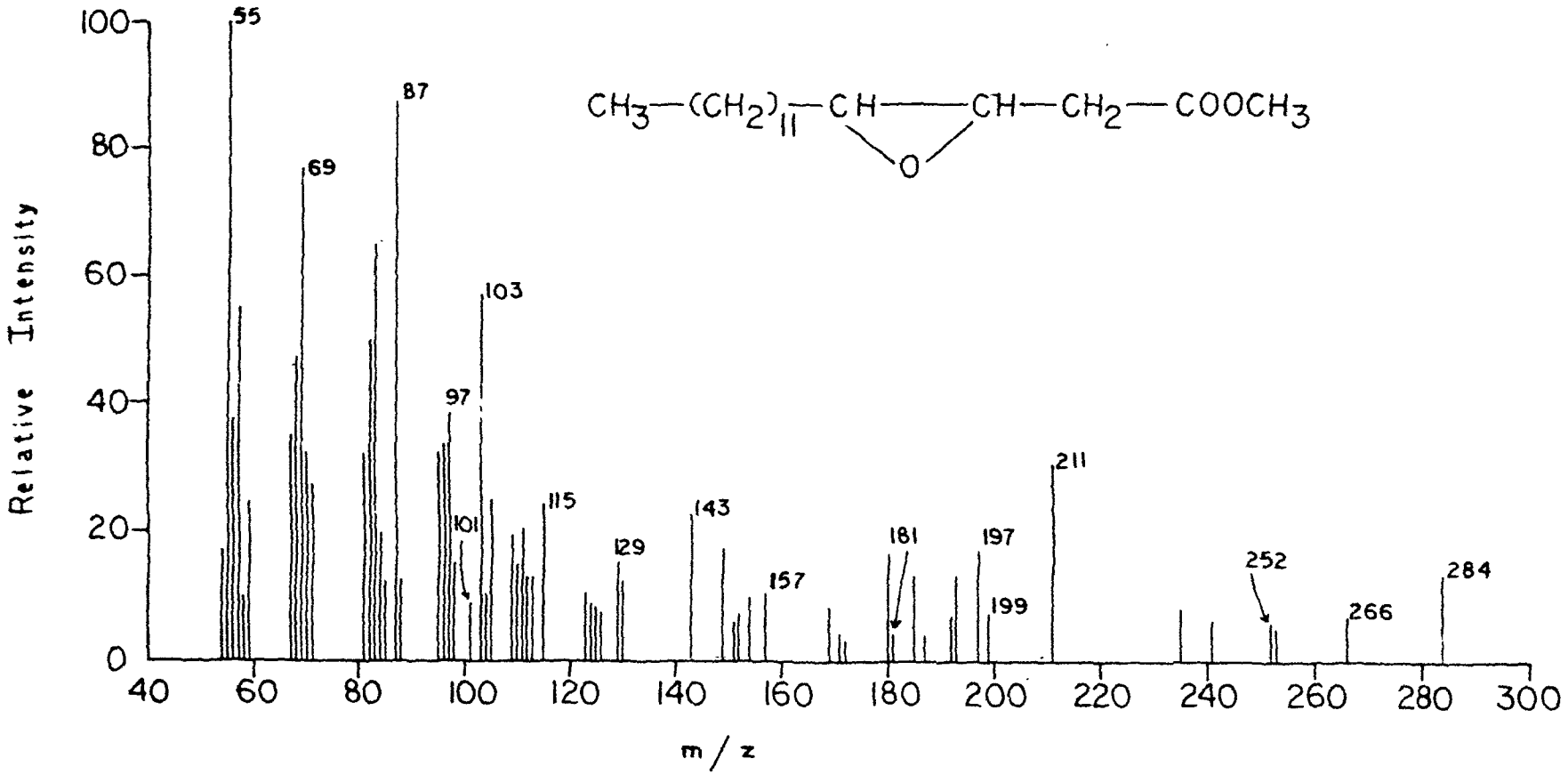


FIG. 3. MS of LVIII

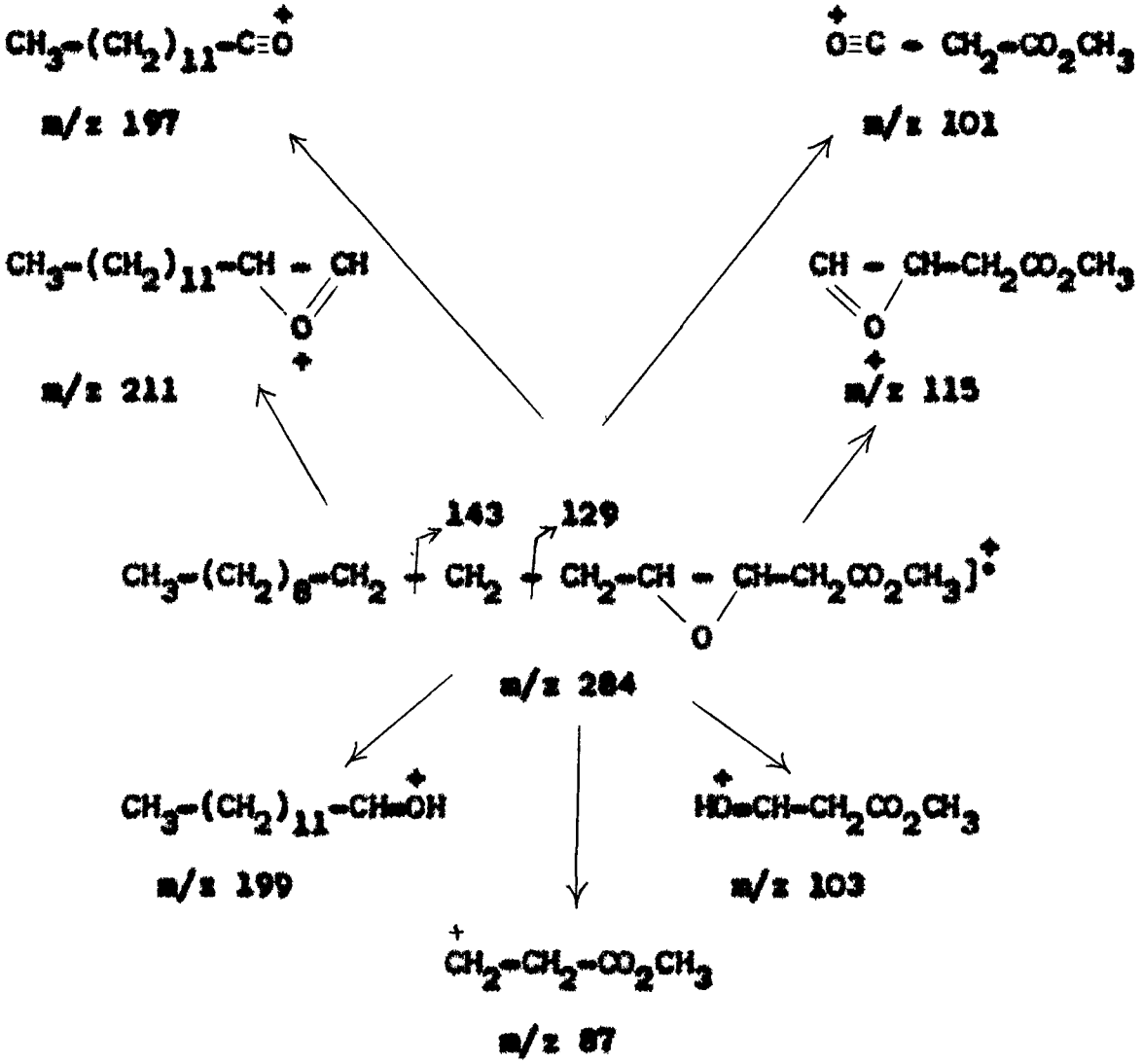


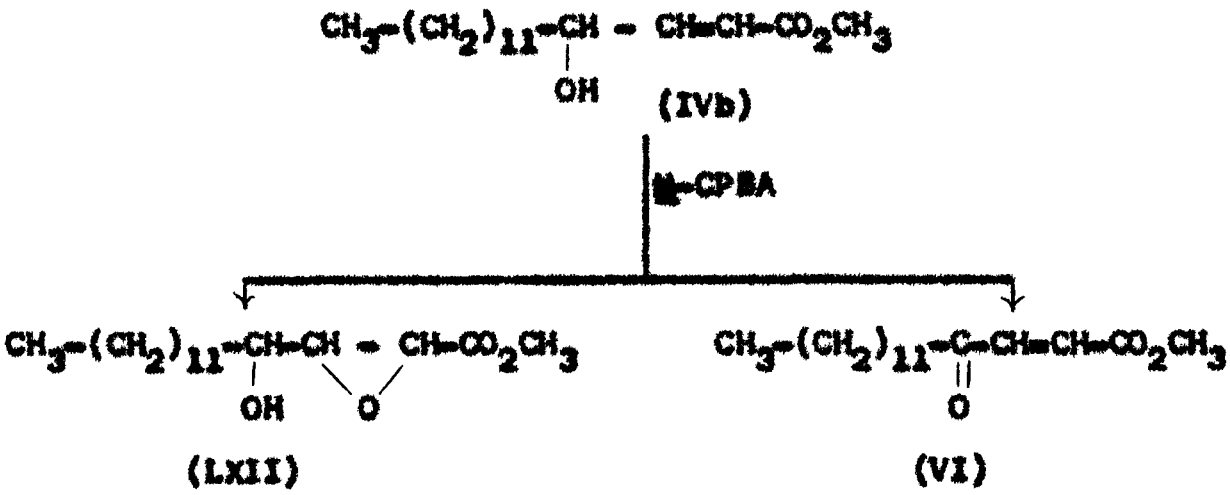
Chart 2. Mass Fragmentation of LVIII.

3.28. Peracid Oxidation of Methyl 4-hydroxy-trans-2-hexadecenoate (IVb)

Compound IVb on epoxidation with m-CPBA (1 mol) at room temperature (10 days) resulted in the formation of a single product LXII (~ 23%). On the other hand refluxing the

reaction mixture after keeping 48 hr. at room temperature yielded LXII (~ 32%). A similar reaction with 2 mol of m-CPBA gave two products: LXII (~ 65.8%) and VI (~ 15.3%). The reaction is schematically represented in scheme 13.

Scheme 13



Characterization of Compound LXII

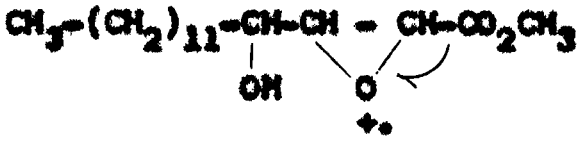
The compound LXII obtained as a white solid (m.p.48-49C) analyzed for $C_{17}H_{32}O_4$. Picric acid[69] gave negative test probably due to the steric hindrance which does not allow the formation of hydroxy picryl ether adduct. Its IR spectrum gave characteristic bands at 3380-3290 (OH), 1730 ($\underline{COO}CH_3$), 1275 and 1230 (C-C and C-O bonds ring breathing)[66], 1120, 1040, (C-O), 880 sharp and 830 cm^{-1} (epoxy ring). The NMR spectrum further confirmed the structure as methyl 4-hydroxy-trans-2,3-

epoxyhexadecanoate (LXII) by displaying characteristic signals at τ 6.25 s (3H, $-CO_2CH_3$), 6.28 br,m (1H, $-\underset{|}{CH}-OH$, merged in parts with the signal at τ 6.25 of methyl ester) and 7.58 br,s (1H, OH, disappeared on addition of D_2O). Two important broad signals appeared at τ 6.6 and 6.8 each integrating for one proton at C_2 and C_3.

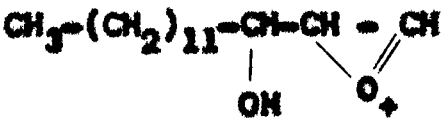
The above formulation was further supported by mass spectrometry. The mass spectrum of compound LXII (Fig.4) gave no molecular ion peak at m/z 300 ($C_{17}H_{32}O_4$) but showed the ions at m/z 282 (300-18), 251 (282-31), 250 (282-32), 241 (M-59), 223 (base peak), 209, 196, 182 (196-14), 168 (182-14), 154 (168-14), 140 (154-14), 136 (168-32), 127 (140-13), 122 (136-14), 114 (127-13), 108 (140-32), 96 (122-26), 94 (108-14), 87, 82 (114-32), 80 (108-28), 70, 68, 66 (80-14), 54 (82-28) and some other ion peaks. The genesis of important fragment ions are discussed below:

<u>m/z 241 (M-59)</u>

This fragment ion can be shown to arise by the loss of mass unit 59 from the molecular ion (α-cleavage).



m/z 300



m/z 241

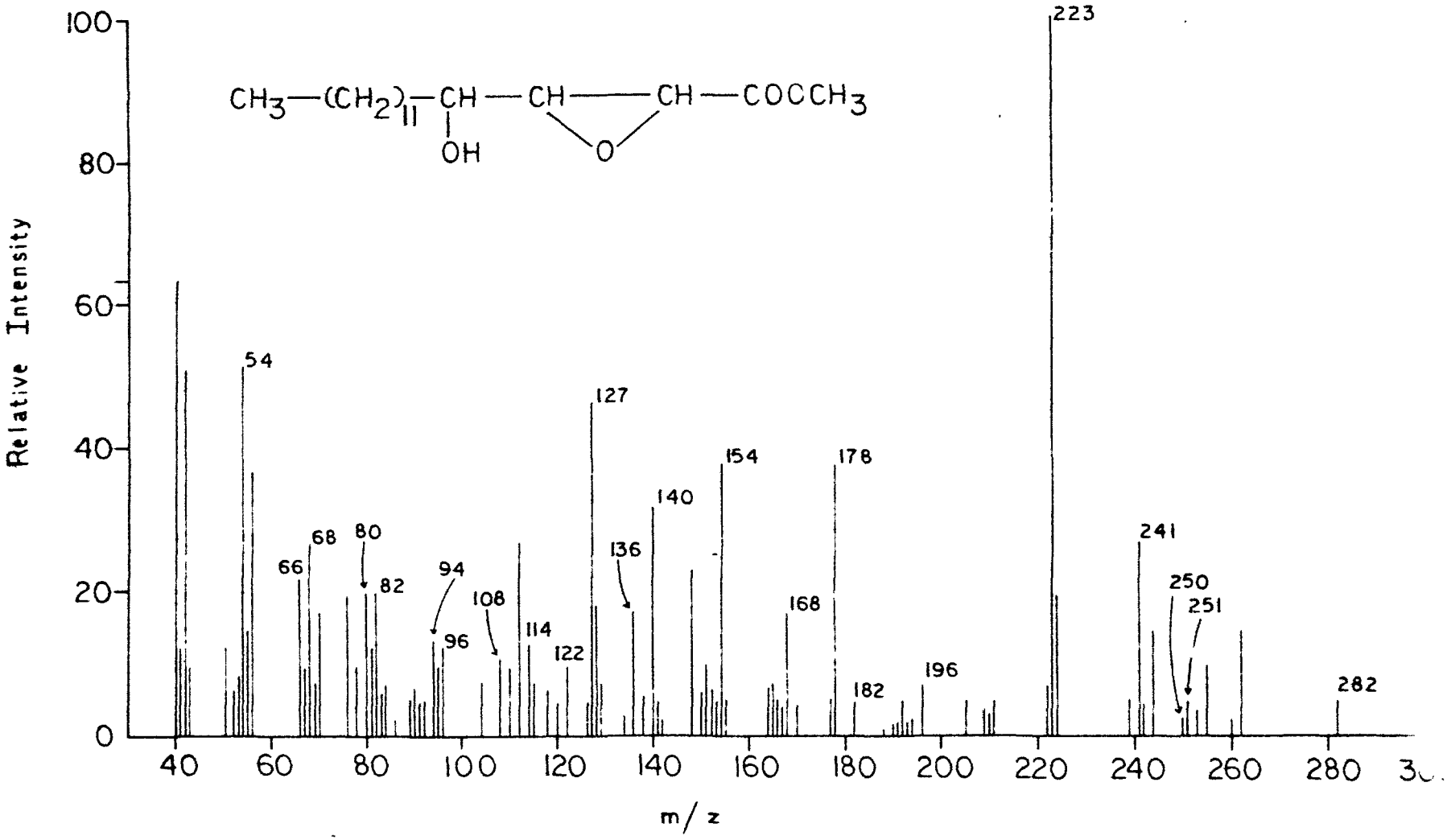
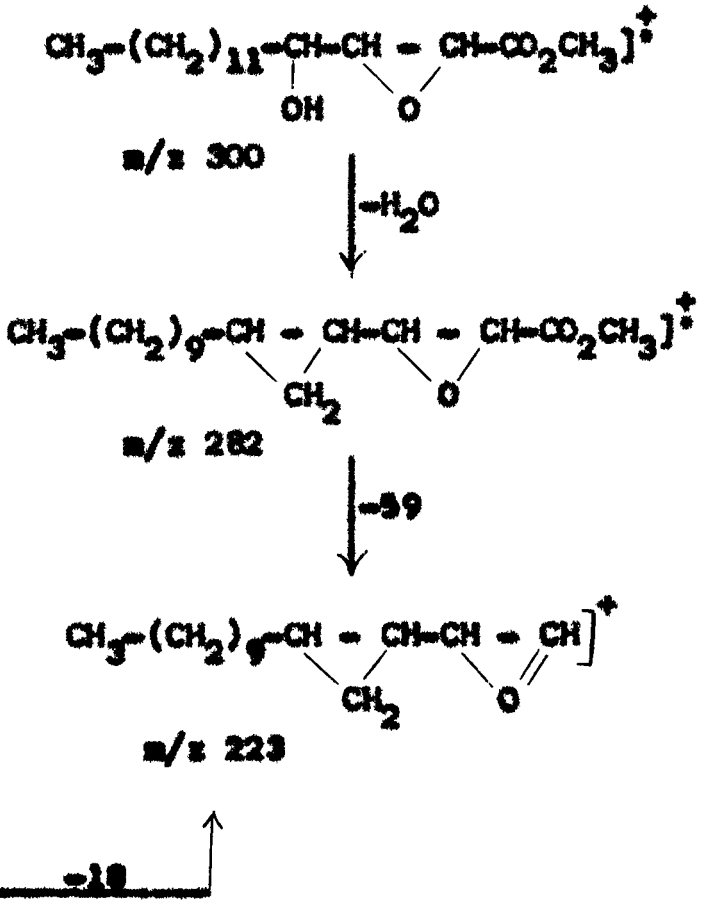


FIG 4 MS of LXII

m/z 223 (282-49 or 241-18)

This is the base peak in the spectrum and the genesis of this fragment ion can be rationalised in two possible ways as under :

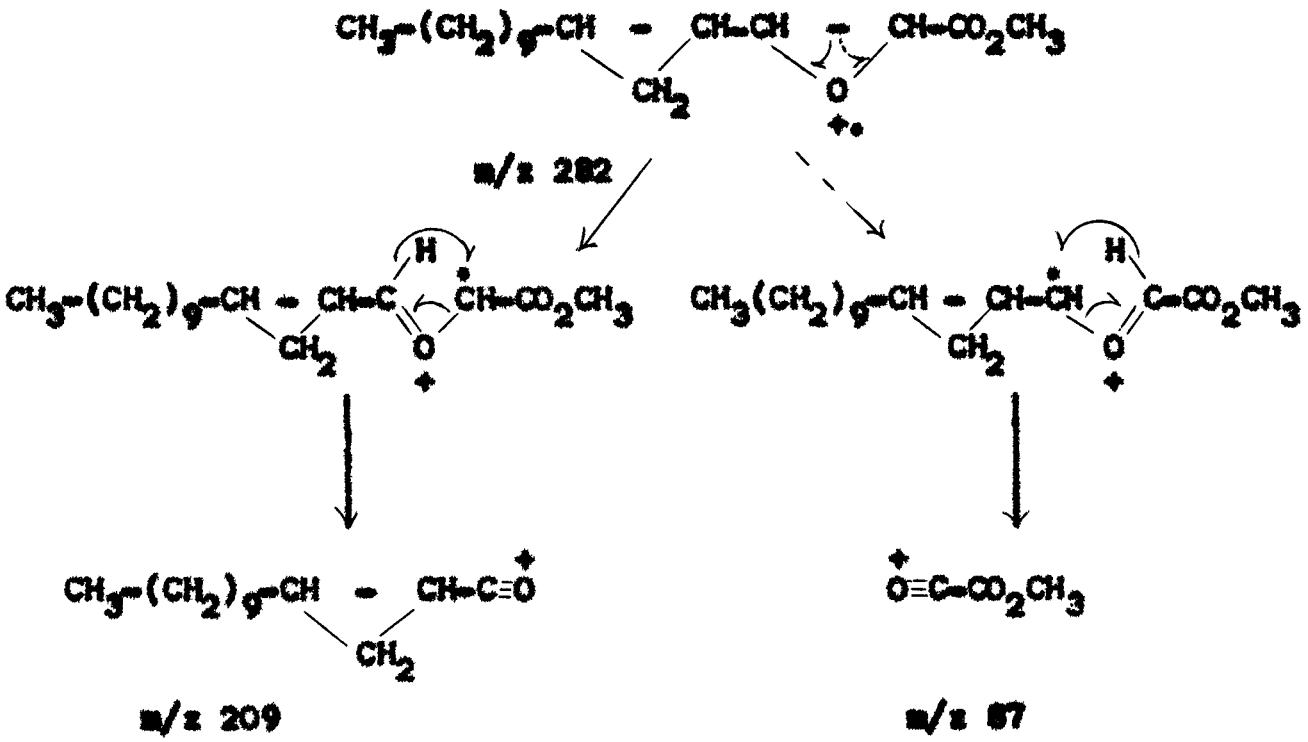
(a) From m/z 282



(b) m/z 241 —— -18 ——↑

m/z 209 (282-73) and 87 (282-195)

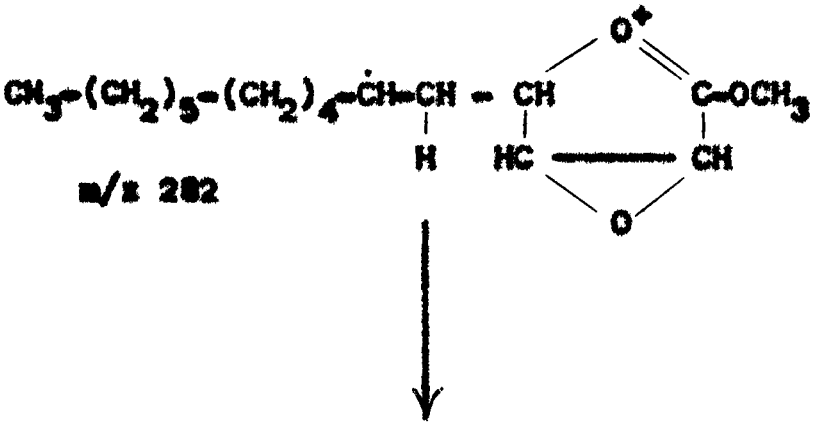
The fragment ions m/z 209 and 87 located the position of epoxy group and can be shown to arise from m/z 282.



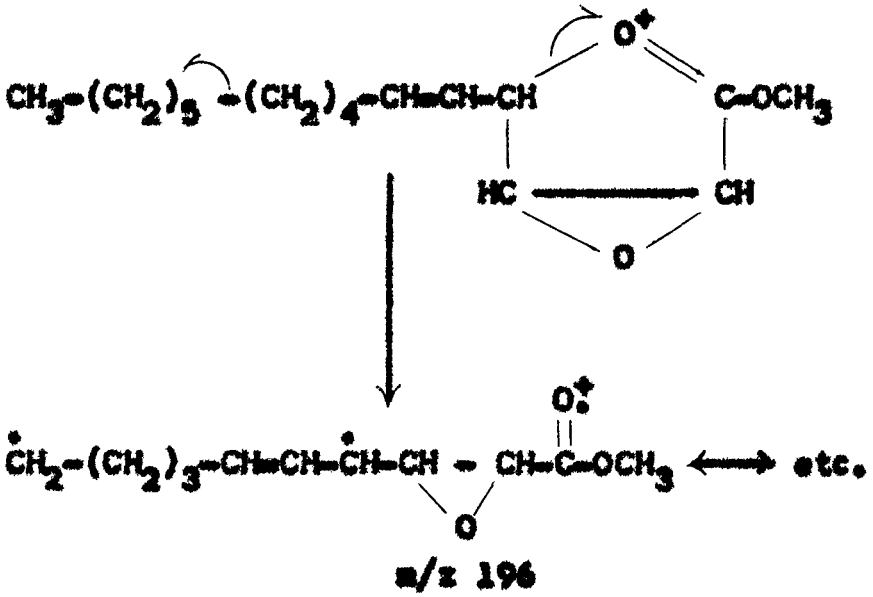
m/z 209 m/z 87

m/z 196 (282-86)

This fragment ion derived from m/z 282 by the loss of m/z 86. A probable mechanism has been proposed to account for the loss of m/z 86.

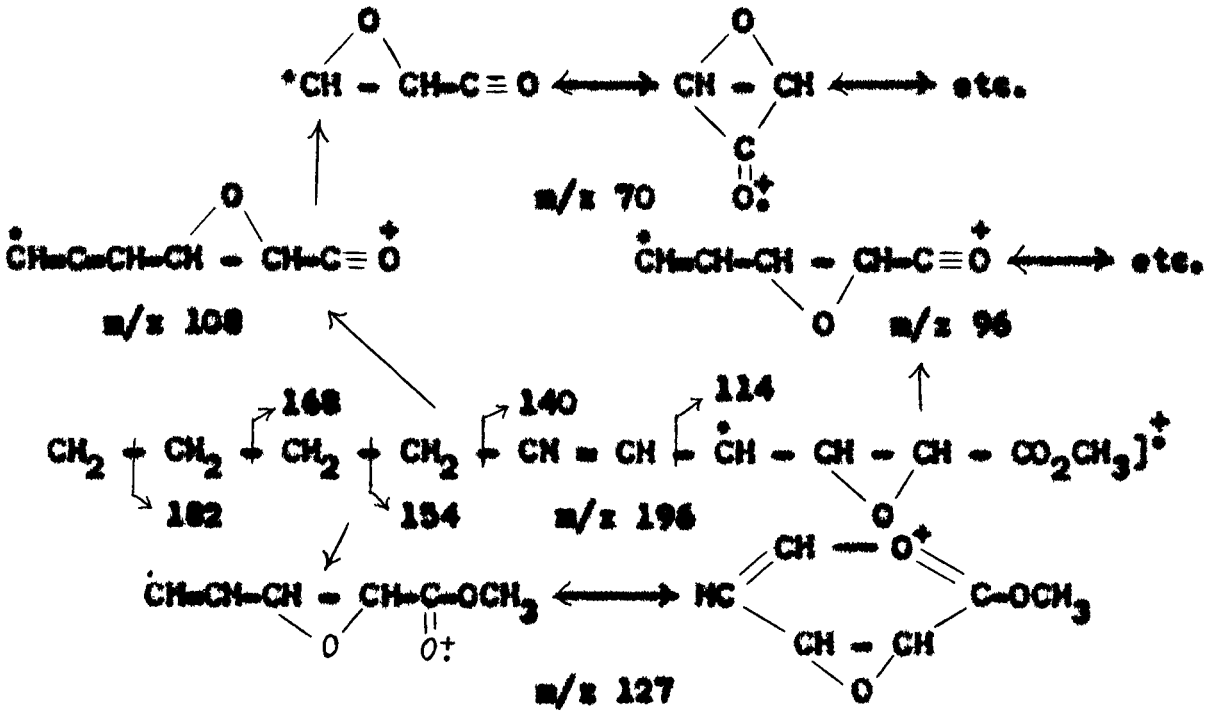


m/z 282



The fragment ions m/z 182, 168, 154, 140, 127 and 114 can be shown as arising from the fragment ion m/z 196. The formation of these fragment ions and their further fragmentation are shown in chart 3.

Chart 3

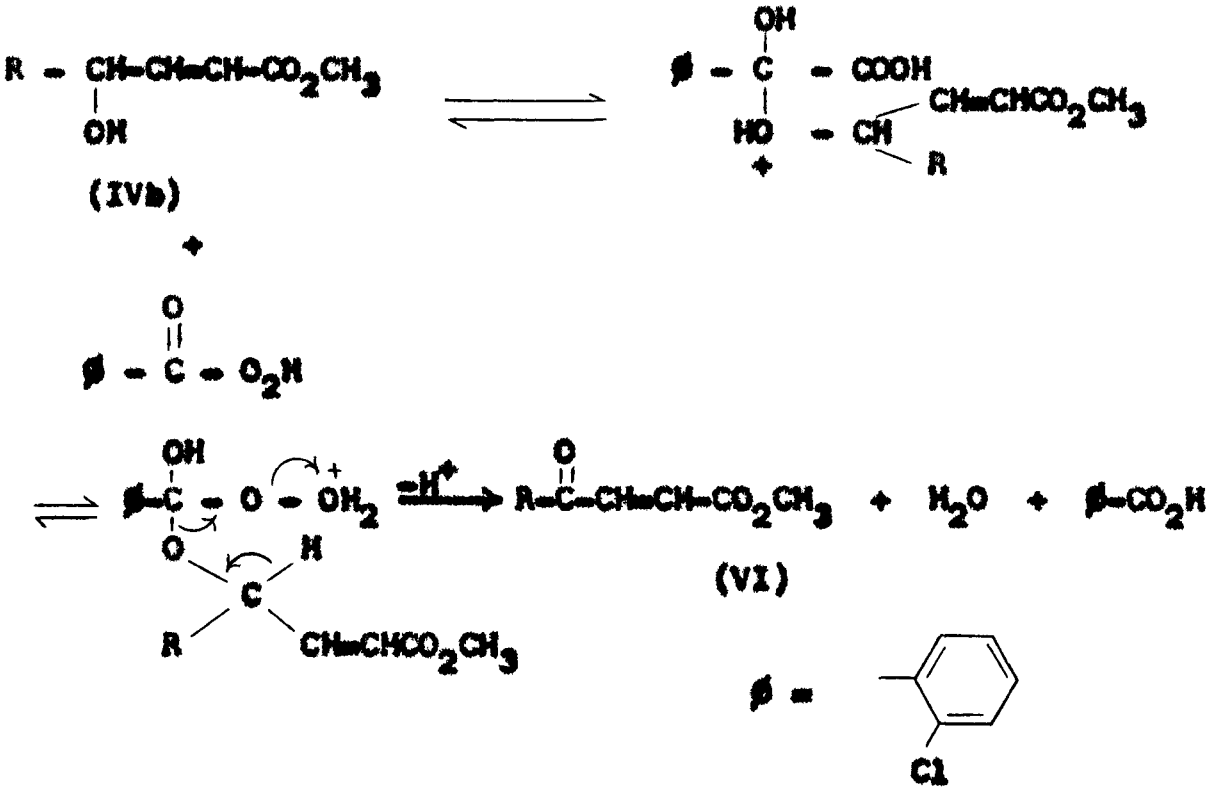


Characterization of Product VI

Elemental analysis of the compound VI corresponded to formula $C_{17}H_{30}O_3$ and gave positive DNP test. This compound had identical UV, IR, NMR and mass spectral values as mentioned in the earlier scheme 11 for VI. Thus product VI was characterized as methyl 4-oxo-*trans*-2-hexadecenoate.

The formation of methyl 4-oxo-*trans*-2-hexadecenoate (VI) during the peracid oxidation of methyl 4-hydroxy-*trans*-2-hexadecenoate (IVb) can be proposed according to scheme 14.

Scheme 14

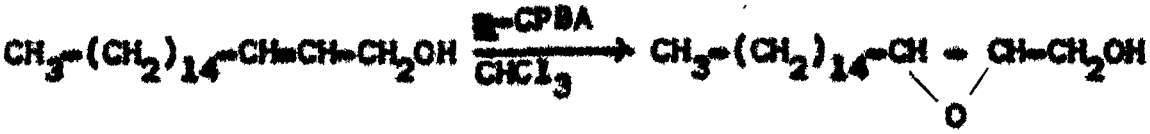


It has been reported[70,71] that peracid reactsslowly with alcohols under ordinary conditions to give ketones. This observation was further supported by Cella et al.[72] that oxidation of secondary alcohols to ketones by m-CPBA occurs readily in presence of catalytic amount of acid. Traces of strong acidic impurity present in commercially available m-CPBA may be responsible for this oxidized product (VI).

3.2C. Peracid Oxidation of trans-2-Octadecen-1-ol (LIV)

Epoxidation of trans-2-octadecen-1-ol (LIV) with m-CPBA yielded a single product (LXIII) which on crystallization from petroleum ether yielded a homogeneous solid (73-74C, yield ~ 100%) (Scheme 15). Although a preliminary report[63] on perphthalic acid oxidation of trans-2-octadecen-1-ol has appeared in 1953, the reaction was more time consuming as compared to the present m-CPBA oxidation. No detail spectral characterization of the resulting product was carried out by previous workers[63]. In order to study the detailed spectral properties of the resulting product and the influence of hydroxyl function on the reactivity of trans-olefinic bond during peracid oxidation, compound LIV was allowed to react with m-CPBA. Pertinent IR, NMR and MS data were used to characterize the compound LXIII.

Scheme 12



(LIV) (LXIII)

Compound LXIII gave positive picric acid test[69] for oxirane oxygen and analyzed for $C_{18}H_{36}O_2$. Its IR spectrum gave bands at 3270, 1450 and 1050 cm^{-1} for hydroxyl group and a sharp band at 860 cm^{-1} for trans-epoxy ring. NMR spectrum displayed diagnostic signals at τ 6.46 m (2H, $-CH_2OH$), 6.52 br,s (1H, $-OH$, D_2O exchangeable) and 7.5 m (2H, $-CH-CH-$, trans-epoxy ring proton). On the basis of microanalysis and spectral data compound LXIII was characterized as trans-2,3-epoxyhexadecan-1-ol.

The MS of LXIII (Fig.5) further supported the structure. It showed small molecular ion peak at m/z 284 along with ions at m/z 283 (M-1), 265 (283-18), 253 (M-31), 241 (M-43), 225 (284-59), 185 (M-99), 184 (185-H), 182 (184-2H), 170 (184-14), 154 (170-16), 152 (170-18), 140 (154-14), 138 (152-14), 124 (138-14), 123 (140-17), 122 (123-H), 110 (124-14), 108 (122-14), 96 (110-14), 94 (140-46), 88, 82 (96-14), 80 (94-14), 74, 73 (M-211), 70 (88-18), 68 (82-14), 66 (80-14), 61, 59, 56 (82-26 or 74-18), 42 (base peak), 40, 38 and 31. Genesis of some significant ions are given in chart 4.

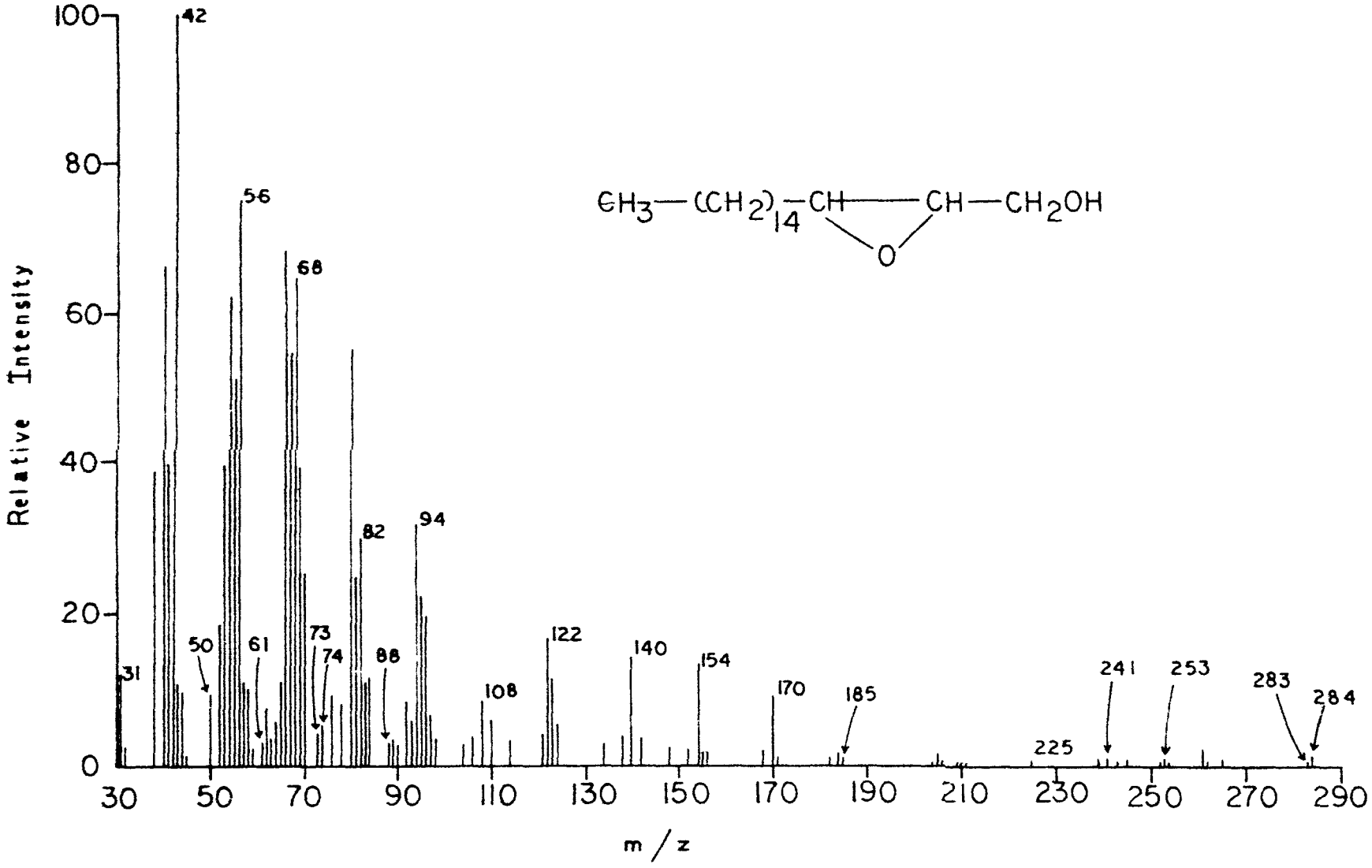


FIG 5. MS of LXIII

Fragment ions m/z 253 and 73 occur from the α-cleavages of epoxy function. In addition trans annular fragmentation takes place with concomitant hydrogen transfer to give fragments at m/z 241 and 61. Characteristic rearrangement ions of epoxy function were observed at m/z 239, 225, 88, 74, 59 and 45.

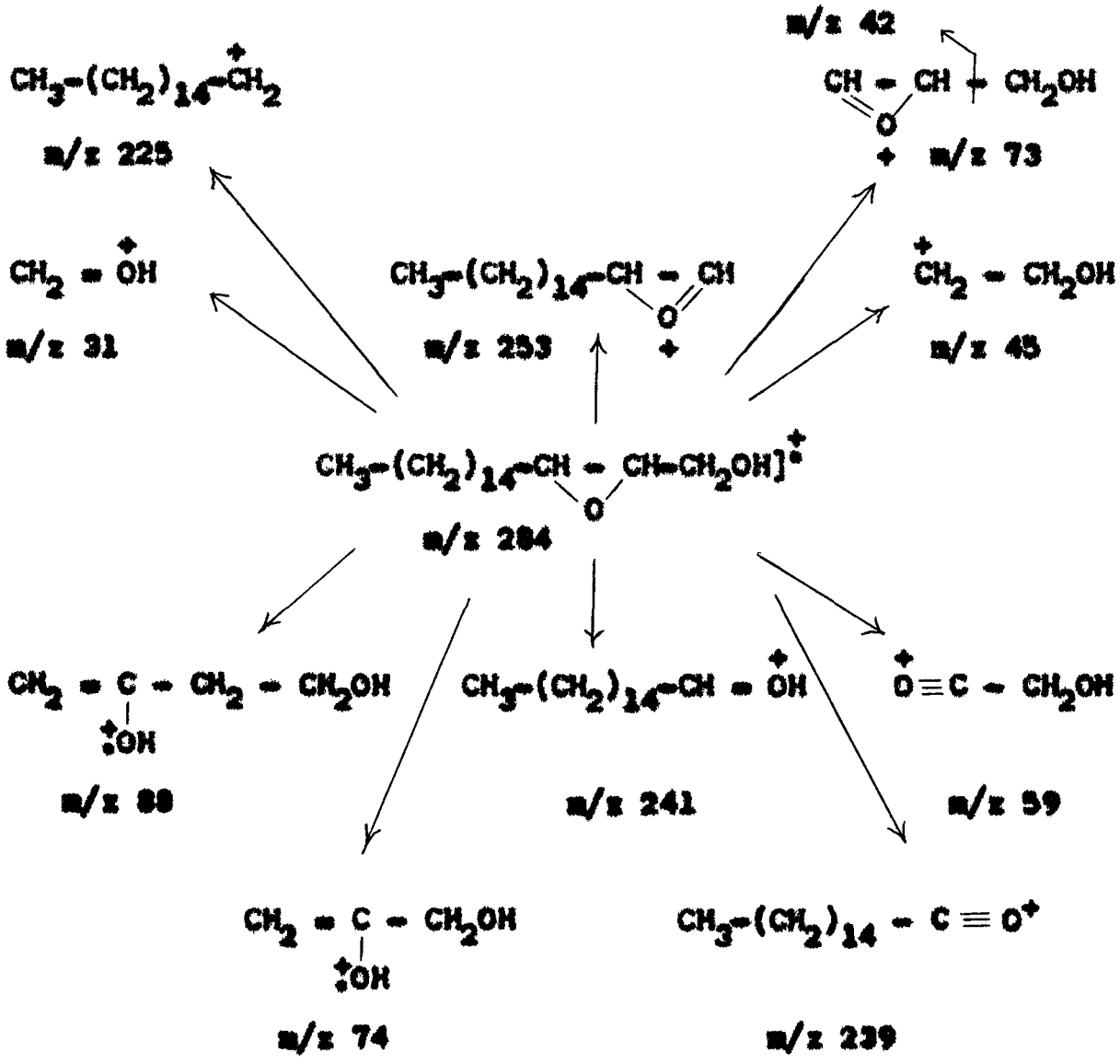


Chart 4. Mass Fragmentation of LXIII

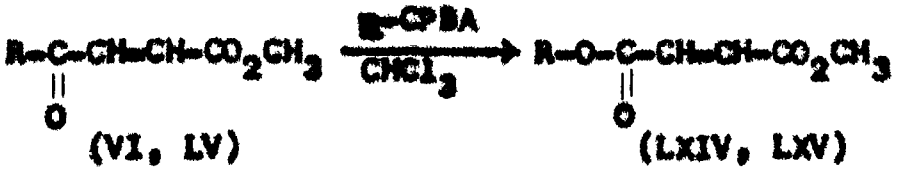
These fragment ions confirm the position of epoxy group at C_2-C_3. A much significant ion at m/z 31 arises from the cleavage of carbon-carbon bond next to oxygen atom showing the presence of terminal hydroxyl function.

It has been observed that peracid oxidation of LIV is easier and quantitative than the ester IVb.

3.2D. Peracid Oxidation of Methyl 4-oxo-*trans*-2-hexa- (VI) and octadecenoate (LV)

Methyl 4-oxo-*trans*-2-hexadecenoate (VI) and methyl-4-oxo-*trans*-2-octadecenoate (LV) were oxidized with m-CPBA. Examination of the final reaction mixture showed a single spot on TLC at a higher R_f value than the original compound. The reaction mixtures on workup yielded corresponding liquid products (LXIV, LXV) (Scheme 16).

Scheme 16



VI, LXIV; R = CH_3-$(CH_2)_{11}$-

LV, LXV ; R = $CH_3(CH_2)_{13}$-

The liquid products were characterized as 1-(methylcarboxy)-2-(dodecylcarboxy) ethylene (LXIV) and 1-(methylcarboxy)-2-(tetradecylcarboxy) ethylene (LXV) by microanalysis as well as IR, NMR and MS spectral studies. These products (LXIV and LXV) exhibited the elemental composition $C_{17}H_{30}O_4$ and $C_{19}H_{34}O_4$, respectively. Since IR and NMR spectra are consistent with the same structure, it is convenient to discuss them together. The IR spectra gave bands at 1735, 1725, 1710 ($-CH_2-O-\overset{O}{\overset{\|}{C}}-CH=CH-\overset{O}{\overset{\|}{C}}-OCH_3$), 1645 ($-HC=CH-$) and 975 cm^{-1} (trans-unsaturation). The other bands were observed at 1295, 1255 (asymmetric C-C-O), 1220, 1165, 1150 (C-O-C) and 1105, 1030, 1005 cm^{-1} (C-O). There is a complete absence of band in the region of 800-900 cm^{-1} showing the absence of epoxy group. NMR spectra were more informative regarding their structures and gave signals at τ 3.21 s (2H, $-\overset{O}{\overset{\|}{C}}-\underset{}{CH}=\underset{}{CH}-\overset{O}{\overset{\|}{C}}-$) and 5.87 t (2H, $-\underset{}{CH_2}-O-$, J=6 Hz) along with usual peaks.

The structure of LXIV was further substantiated by its mass spectrum (Fig.6). It gave peaks at m/z 299 (M+1), 298 ($M^{\cdot}$), 267 (M-31), 266 (M-32), 249 (267-18), 239 (M-59), 238 (239-H), 237 (238-H), 225 (238-13), 224 (237-13), 222, 221 (237-26), 213 (M-85), 211, 210 (237-27), 209, 185 (M-113), 168 (211-C_3H_7), 158 (185-CH_2O), 154 (155-H or 168-14), 153 (154-H), 140 (M-158 or 154-14), 132, 131 (M-167), 130, 126, 125, 115, 114 (131-17 or M-184), 113 (M-185, base peak), 112 (130-18 or

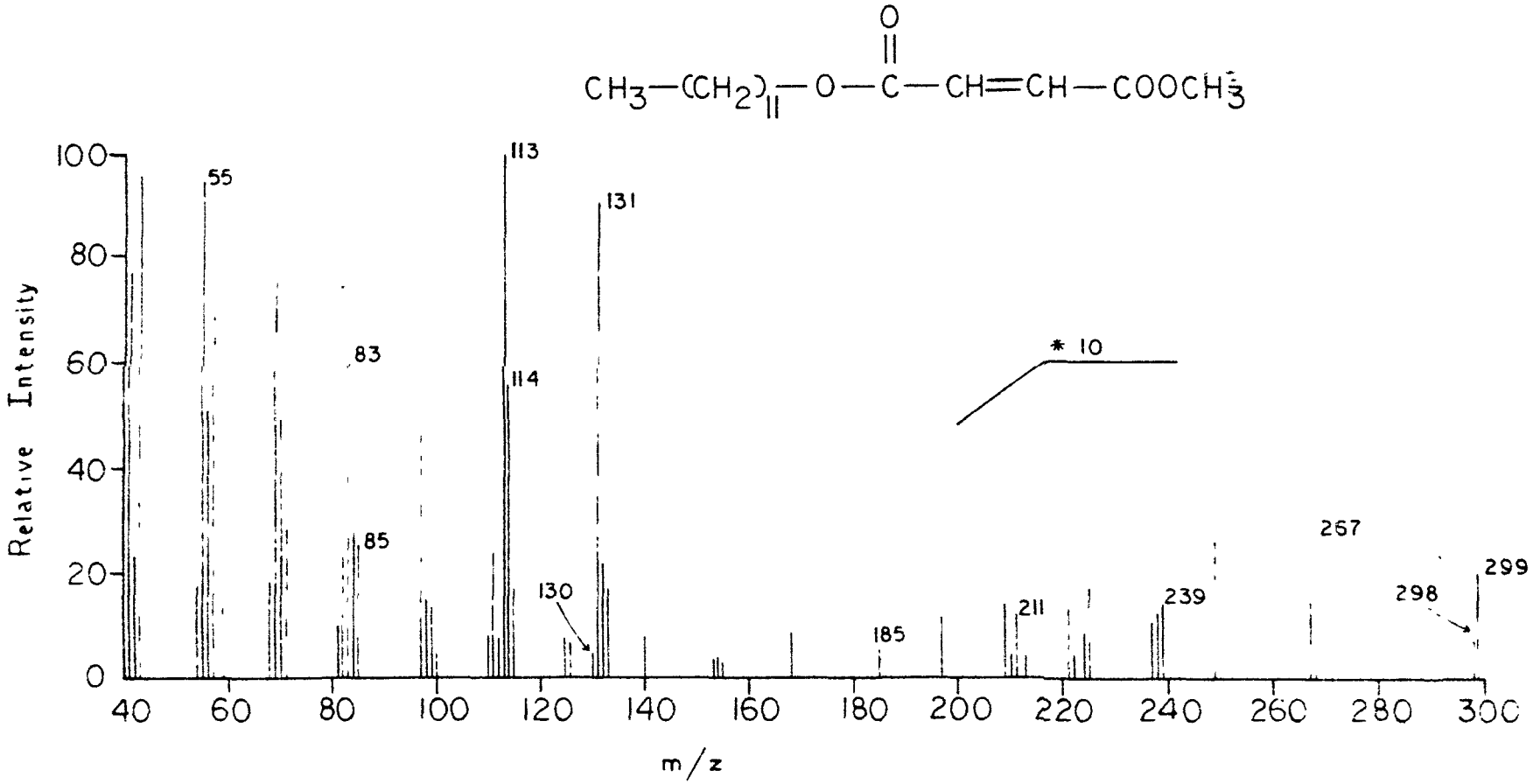


FIG 6 MS of LXIV

113-H), 111, 110, 100 (131-31), 99 (130-31), 98 (130-32), 97, 85 (113-28), 83 (114-31), 82 (114-32 or 113-31), 81 (113-32), 71 (130-59), 70, 69, 59, 57, 56, 55 and 54 (113-59). The formationsof some structure revealing fragment ions are given in chart 5.

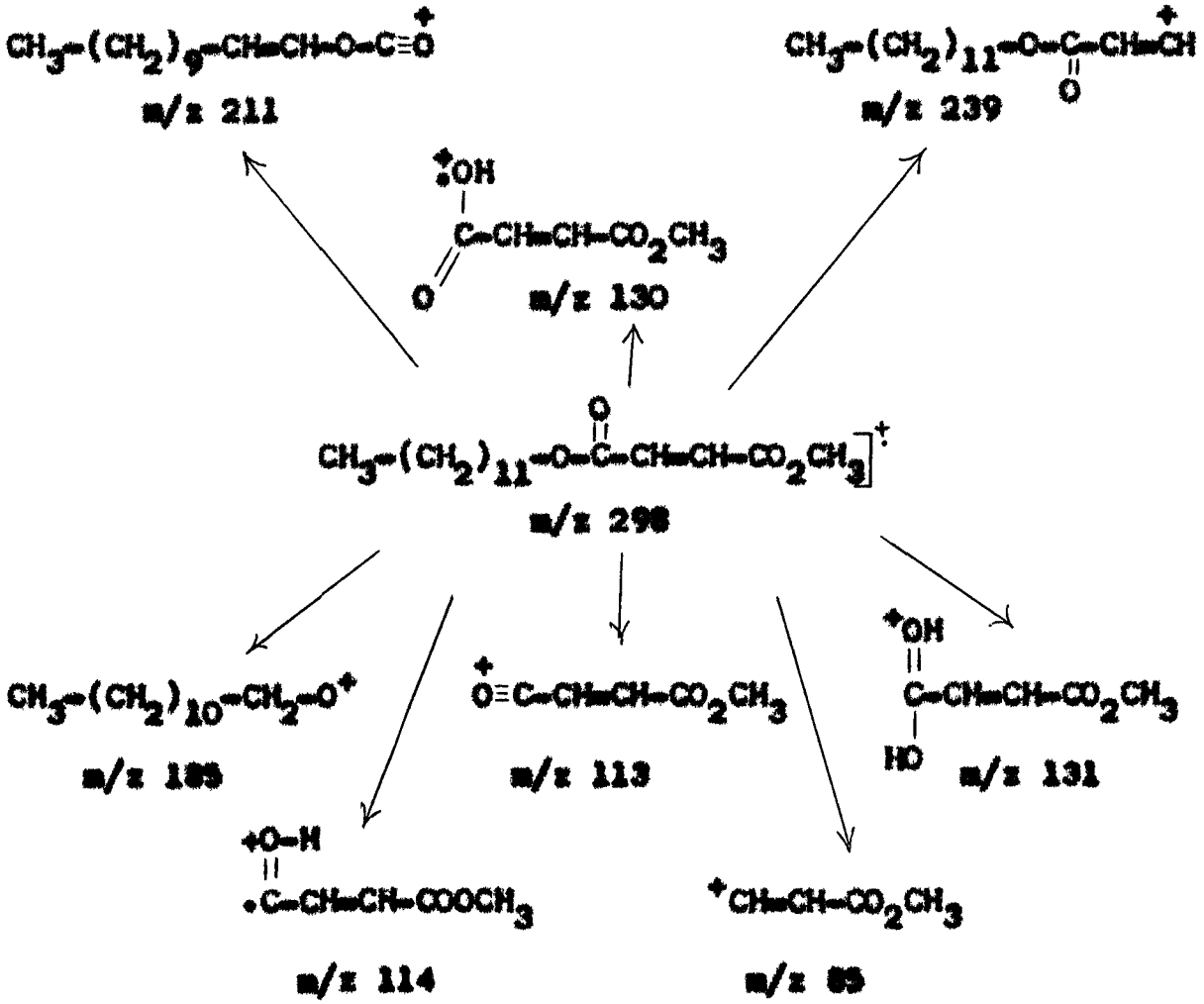
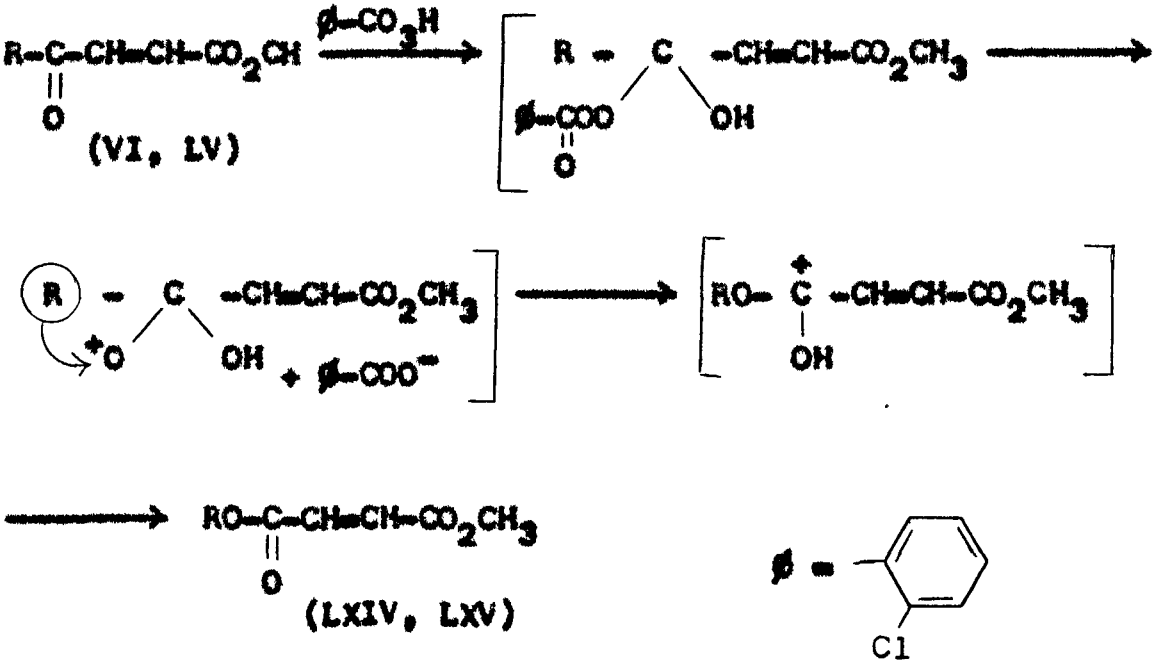


Chart 5. Mass Fragmentation of LXIV

Fragment ions 185 and 113 originate by the α-cleavage to C_4-oxo group. The fragment ion m/z 113 of higher intensity constitutes the base peak. These fragments show that insertion of oxygen takes place between C_4 and C_5. The fragment ions 82, 81 and 54 further support the formation of ion m/z 113.

It is expected that the compounds LXIV and LXV are formed from VI and LV via the Baeyer-Villiger oxidation. Because the olefinic character of α,β-unsaturated ester is further reduced due to the presence of oxo-group at C_4. To support this statement it can be mentioned that α,β-unsaturated ketone[73] and non-cyclic acetals[74] give the corresponding unsaturated esters. The mechanism for the formation of these products can be suggested as indicated below:



The peracid first adds to the carbon-oxygen double bond; then the oxygen-oxygen bond is heterolytically cleaved. Preferential migration of bulky alkyl group from carbon to oxygen thus takes place leading to the formation of the di-esters (LXIV, LXV).

4. Quantitative Ring Cleavage of Long-Chain Epoxides by Chlorotrimethylsilane (CTMS)

It is well known that the epoxide or oxirane ring undergoes a wide variety of ring-opening reactions with a broad range of electrophiles and nucleophiles. One method for obtaining halohydrin is the reaction of oxirane with halogen halides. Several publications[75] have highlighted diverse utility of CTMS [$(CH_3)_3SiCl$] as an important reagent for organic synthesis. This reagent can be used either directly or in combination with a suitable metal. The versatile nature of the CTMS in chemical transformation prompted us to carry out the reactions of some epoxy fatty compounds with this reagent.

4.1. Preparation and Isolation of Epoxy Compounds

4.1A. Methyl 10,11-epoxyundecanoate (LXVI)

Using m-CPBA the methyl 10-undecenoate was epoxidized to methyl 10,11-epoxyundecanoate (LXVI). The column purified epoxy ester (LXVI) gave positive colour reaction with picric acid TLC. Its IR spectrum gave bands at 1740 and 835 cm^{-1} for ester carbonyl and epoxy groups, respectively. The NMR spectrum showed three proton multiplet centered between τ 7.4-7.6 which was indicative of the protons attached to the oxirane oxygen.

4.1B. Methyl cis-9,10-epoxystearate (LXVII)

Compound LXVII was prepared from methyl cis-9-octadecenoate using m-CPBA as oxidant. The epoxyester (LXVII) purified through silica gel column showed in its IR spectrum, band at 1740 cm^{-1} for ester carbonyl, the moderately intense bands at 840 and 820 cm^{-1} for cis-epoxide group. The NMR spectrum showed signals at τ 7.31 m (2H, $-\underset{\diagdown O \diagup}{CH} - CH-$), 7.78 m ($2H, -CH_2-CO_2CH_3$), 8.68 br,s (chain $-CH_2-$) and 9.12 t ($3H, CH_3$).

4.1C. Methyl trans-2,3-epoxyhexadecanoate (LVIb) and trans-2,3-Epoxyoctadecan-1-ol (LXIII)

These epoxy compounds were prepared as described in chapter 3.

4.1D. Methyl cis-12,13-epoxy-cis-9-octadecenoate (LXVIII)

The naturally occurring cis-olefinic epoxyester (LXVIII) was isolated in the pure form from Vernonia anthelmintica seed oil. Its IR spectrum gave bands at 1740 (ester carbonyl) and 840, 820 (cis-epoxy group). The NMR spectrum gave characteristic signals at τ 4.58 m (2H, $-CH=CH-$), 7.31 m (2H, $-\underset{\diagdown O \diagup}{CH} - CH-$), 7.78 m (4H, $-\underset{\diagdown O \diagup}{CH} - CH-CH_2-CH=CH-(CH_2)_6-CH_2-CO_2CH_3$, a part merged with the signal at τ 7.94) and 7.94 m ($2H, -CH=CH-CH_2-$).

4.2A. Reaction of Methyl 10,11-epoxyundecanoate (LXVI) with CTMS

Reaction of LXVI with an ethereal solution of CTMS yielded a crystalline product (LXIXa, m.p.47-48C) (Scheme 17). The product gave a single spot on TLC in different solvent systems. It was analyzed for $C_{12}H_{23}O_3Cl$ and gave positive Beilstein test. Exclusive formation of methyl 11-chloro-10-hydroxyundecanoate (LXIXa) rather than its isomer, methyl 10-chloro-11-hydroxyundecanoate (LXIXb) was supported by spectral data. The IR spectrum showed bands at 3360 (OH), 1730 ($\underline{COO}CH_3$) and 730 (C-Cl). Its NMR spectrum exhibited bands at τ 6.2-6.5 br,m (3H, $\underset{Cl}{CH_2}$ - $\underset{OH}{CH}$-, a part merged with ester proton signal at τ 6.3) and 6.78 s (1H, O$\underline{H}$, disappeared on addition of D_2O).

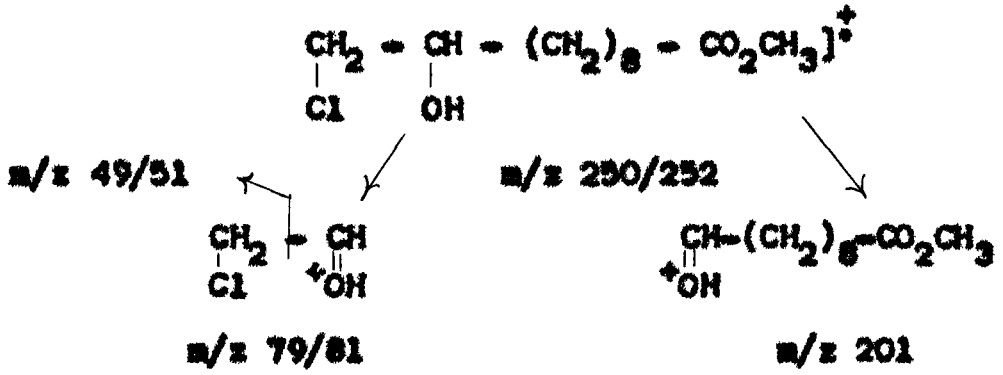
Scheme 17

$$R - CH - CH - R' \xrightarrow[\text{ether}]{CTMS} R - \underset{Cl/OH}{CH} - \underset{OH/Cl}{CH} - R'$$

(LXVI-LXVIII, LVIb, LXIII) (LXIX-LXXIII)

Compound	R	R'
LXVI, LXIXa,b	H	$-(CH_2)_8-CO_2CH_3$
LXVII, LXX	$CH_3-(CH_2)_7-$	$-(CH_2)_7-CO_2CH_3$
LVIb, LXXIa,b	$CH_3(CH_2)_{12}-$	$-CO_2CH_3$
LXIII, LXXII	$CH_3(CH_2)_{14}-$	$-CH_2OH$
LXVIII, LXXIII	$CH_3(CH)_4-$	$-CH_2-CH=CH-(CH_2)_7-CO_2CH_3$

Mass spectrum of LXIXa (Fig.7) was more helpful in confirming its structure. Structure-revealing peaks were observed at m/z 251/253 (M+1), 233/235 (M-17), 219/221 (M-31), 201 (M-CH_2Cl), 183 (201-18), 170 (201-31), 169 (201-32), 151 (169-18 or 183-32), 124 (183-59), 123 (183-60), 79/81 and 74 (McLafferty rearrangement). The intense peak at m/z 201 established that hydroxyl group is attached with C_{10}.



From the foregoing spectral data it was concluded that the compound LXIXa is methyl 11-chloro-10-hydroxyundecanoate.

The formation of only one isomer (LXIXa) in the ring-opening of methyl 10,11-epoxyundecanoate (LXVI) indicated that the reaction is regiospecific. The electrophilic silicon which attacks the oxirane oxygen to give onium species (C) weakens the C-O bond of oxirane. Consequently the positive charge on the carbon atom increases. This intensified partial positive charge enables the chloride ion to attack the oxirane ring from the back side as illustrated below (Scheme 18).

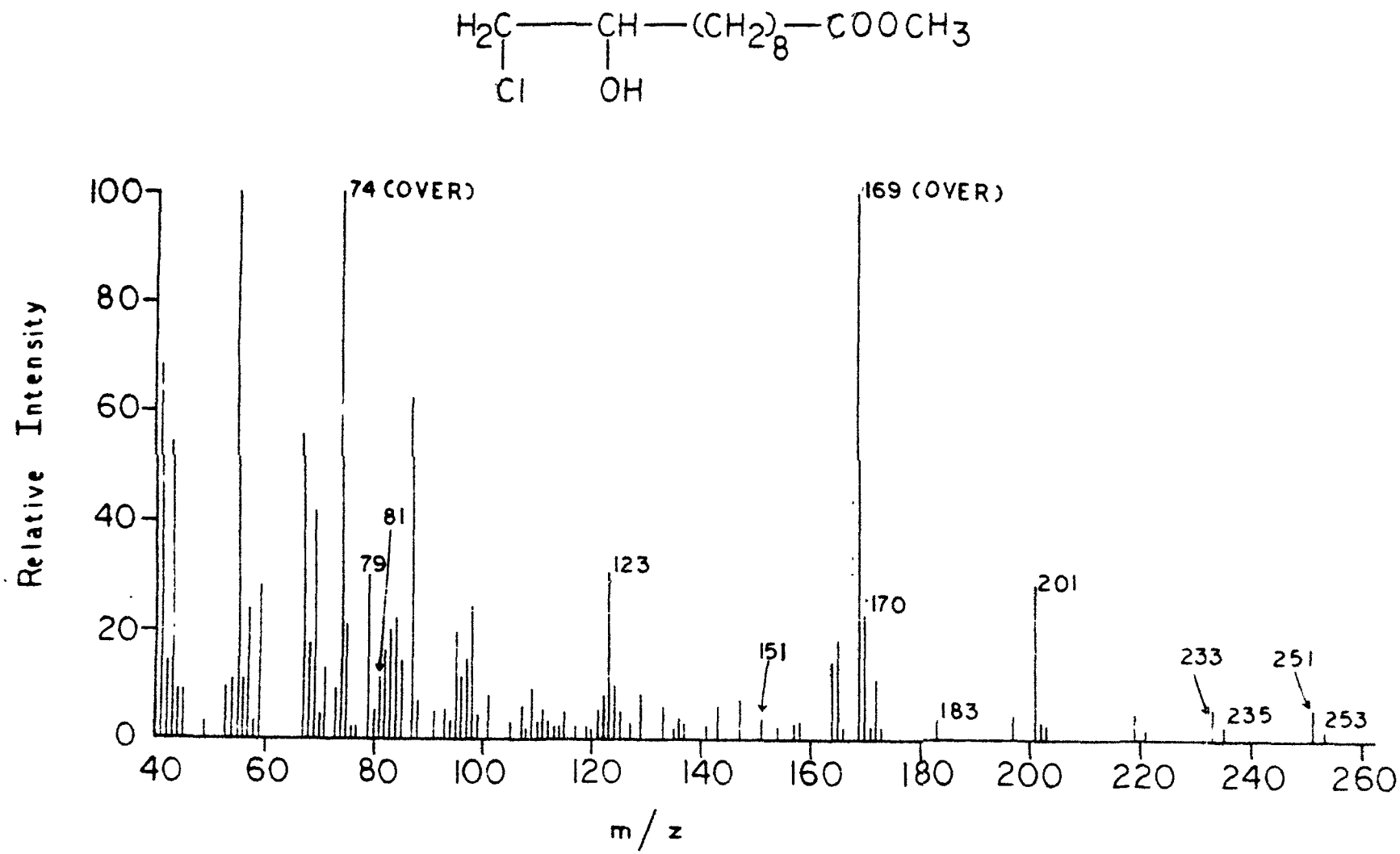
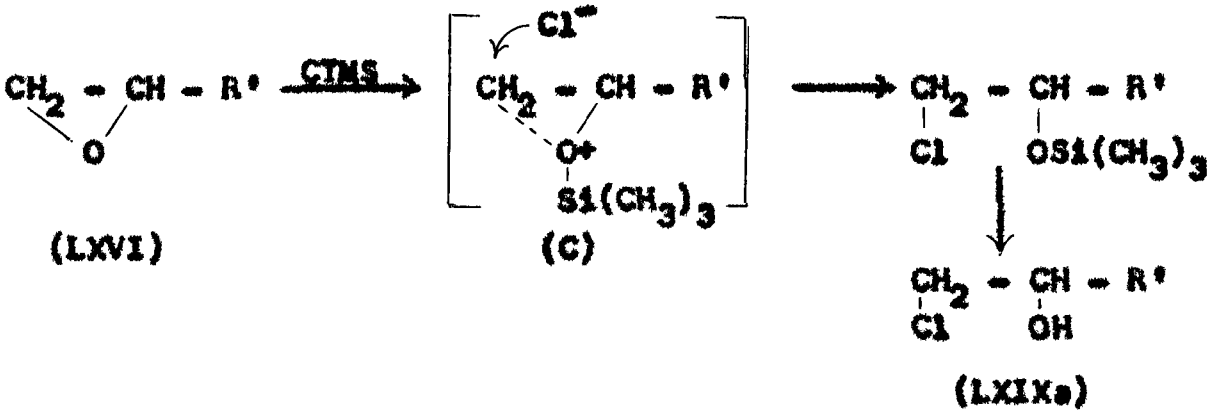


FIG 7. MS of LXIXa

Scheme 18



(LXVI) (C) (LXIXa)

4.28. Reaction of Methyl cis-9,10-epoxystearate (LXVII) with CTMS

A similar reaction of LXVII with CTMS yielded an oily product LXX. It responded to Beilstein test. It was identified as an isomeric mixture, methyl 9(10)-chloro-10(9)-hydroxyoctadecanoate on the basis of microanalysis and spectral data. Elemental analysis corresponded to formula $C_{19}H_{37}O_3Cl$. IR spectrum exhibited bands at 3440 (OH), 1740 (ester carbonyl) and 720 cm^{-1} (C-Cl). The NMR spectrum gave characteristic signals at τ 6.05 m (1H, -C<u>H</u>-Cl), 6.4 m (1H, -C<u>H</u>-OH), 7.45 s (1H, O<u>H</u>, D_2O exchangeable) and 7.7 m (2H, -C<u>H</u>$_2$-CO_2CH_3). The signals of -C<u>H</u>-Cl and -C<u>H</u>-OH are found merged in part with the ester proton signal at τ 6.32.

4.2C. Reaction of Methyl trans-2,3-epoxyhexadecanoate (LVIb) with CTMS

Reaction of LVIb with CTMS yielded a mixture of chlorohydroxy derivatives as shown by two distinct spots visible on TLC. Fractionation of the product on a silica gel column yielded two products, the liquid one (LXXIa, minor) and other solid (LXXIb, major, m.p.40C).

Characterization of Product LXXIa

Combustion data corresponded to formula $C_{17}H_{33}O_3Cl$. Its IR spectrum showed absorptions at 3470 (OH), 1735($\underline{C}OOCH_3$) and 825 cm^{-1} (C-Cl). The NMR spectrum gave the significant signals at τ 5.30 (1H, -C$\underline{H}$-Cl), 5.9 (1H, -C$\underline{H}$-OH), 6.1 s (3H, $-CO_2C\underline{H}_3$) and 7.12 br,s (1H, -CH-O$\underline{H}$). The signals of protons attached to chlorine and hydroxyl-bearing carbons appeared as unresolved multiplets. The methine signal appeared downfield due to the deshielding effect of the chlorine substituent. Thus compound LXXIa was characterized as methyl 2-chloro-3-hydroxyhexadecanoate.

The above structure was further confirmed by its mass spectrum (Fig.8). The spectrum showed significant peaks at m/z 321/323 (M+1), 303/305 (M-17), 302/304 (M-18), 285 (M-Cl), 284 (M^+-HCl), 261/263 (M-59), 225 (261-HCl), 137/ 139, 108/ 110 and other low mass ions including the base peak at

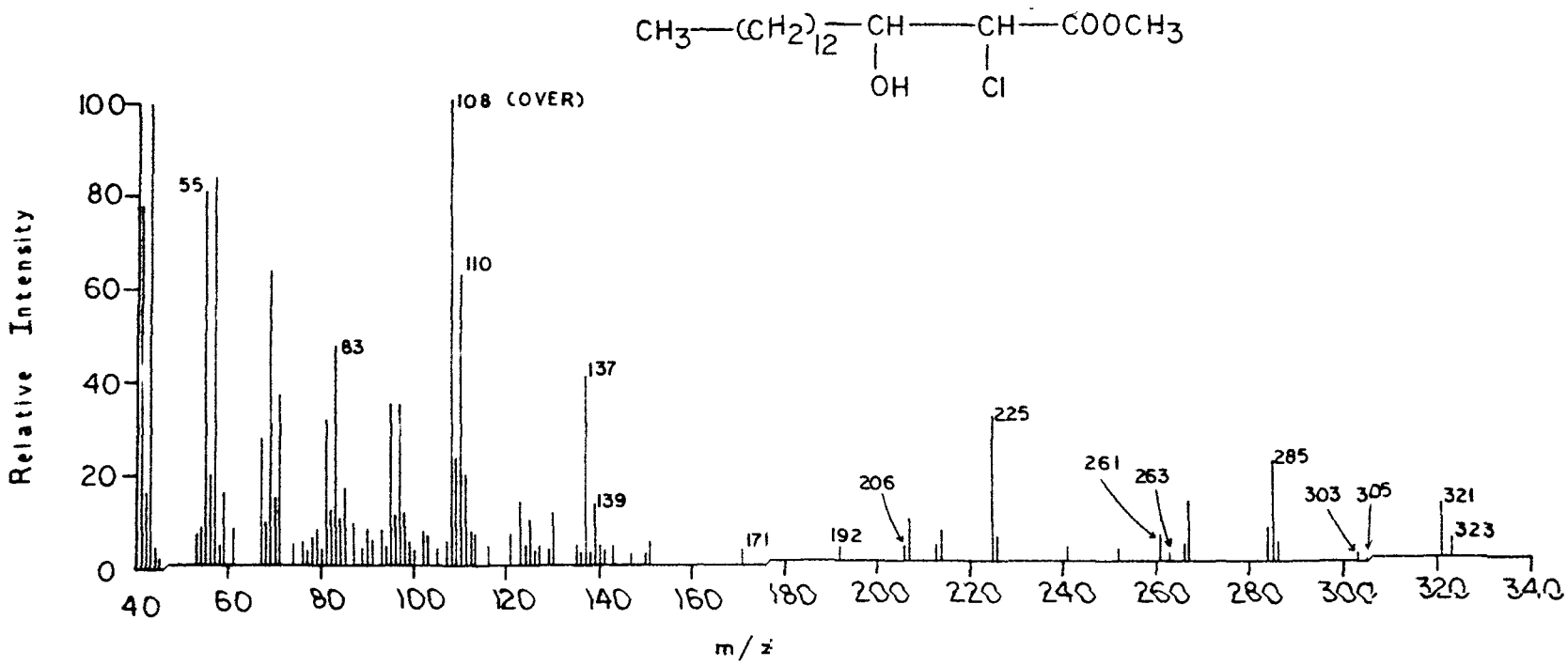


FIG. 8. M S of LXXIa

m/z 43. The appearance of the ion peaks at m/z 137/139 is expected to originate from either of the two isomers (LXXIa) or (LXXIb).

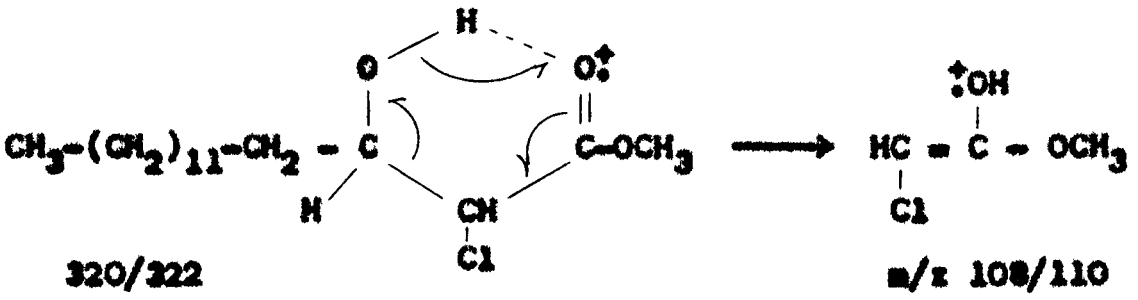
(a) $CH_3-(CH_2)_{12}-CH(OH)-CH(Cl)-CH-CO_2CH_3 \longrightarrow HO=CH-CH(Cl)-CO_2CH_3$

(LXXIa) m/z 137/139

(b) $CH_3(CH_2)_{12}-CH(Cl)-CH(OH)-CH-CO_2CH_3 \longrightarrow CH(Cl^+)-CH(OH)-CO_2CH_3$

m/z 137/139

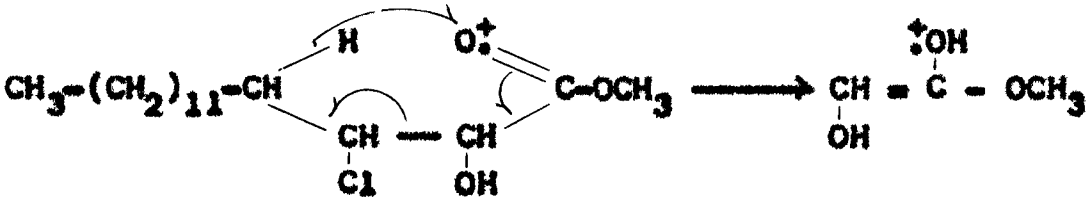
The chlorine-containing fragment ions m/z 108/110 have helped beyond doubt in ariving at the correct structure of the compound LXXIa as methyl 2-chloro-3-hydroxyhexadeca-noate. These fragmentations[13] can be shown either through normal McLafferty rearrangement or according to the scheme indicated under :



Characterization of LXXIb

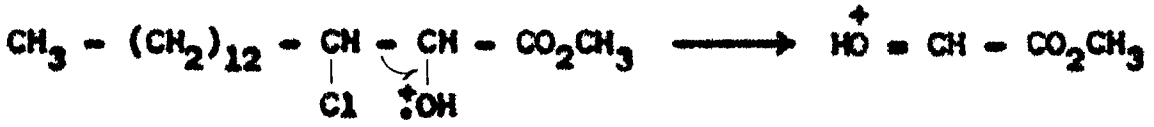
Elemental analysis of LXXIb corresponded to formula $C_{17}H_{33}O_3Cl$. Its IR spectrum exhibited the bands at 3530 (OH), 1740 (ester carbonyl) and 810 cm^{-1} (C-Cl). The NMR spectrum gave important signals at τ 5.8 m (1H, -C<u>H</u>-Cl), 6.0 m (1H, -C<u>H</u>-OH), 6.2 s ($-CO_2C\underline{H}_3$) and 6.9 br,s (1H, -CH-O<u>H</u>, D_2O exchangeable).

The mass spectrum revealed M + 1 peaks at m/z 321/323. The spectrum displayed the same fragmentation pattern as that of 2-chloro-3-hydroxy isomer. The two significant peaks, though of low intensity, were observed at m/z 90 and 89. The appearance of ion at m/z 90 could be due to the McLafferty rearrangement.



m/z 320/322 m/z 90

The ion peak at m/z 89 can be shown to arise as indicated under:

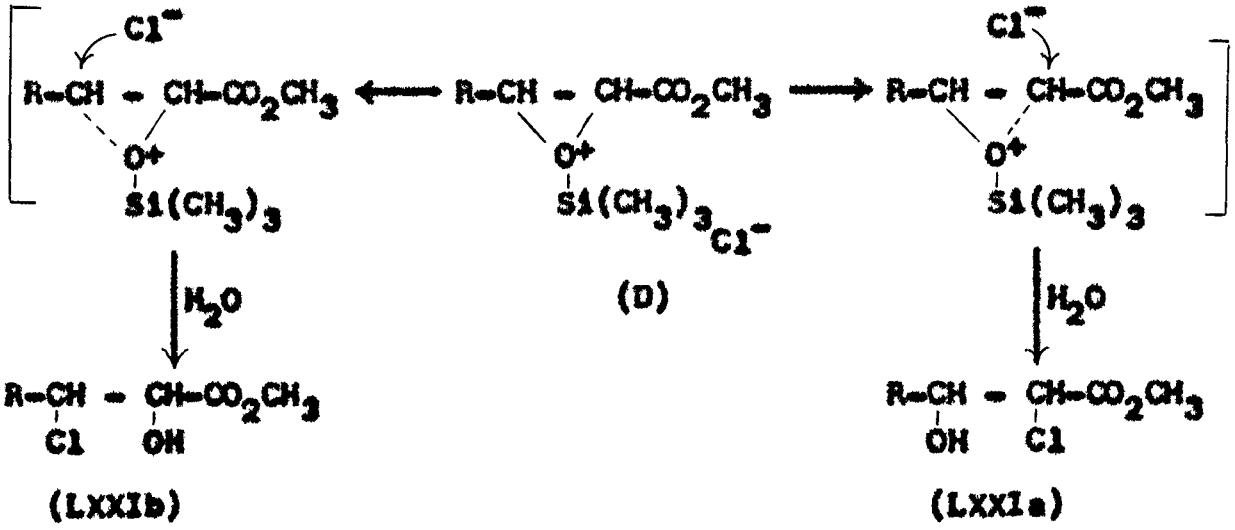


m/z 320/322 m/z 89

From the above data, the product LXXIb was characterized as methyl 3-chloro-2-hydroxyhexadecanoate.

The formation of the major compound LXXIb can be shown in scheme 19. Firstly electrophilic silicon attacks on oxygen to give onium species D. The presence of electron-withdrawing group adjacent to epoxy function probably plays a key role in the opening of onium ion intermediate which accounts for the major yield of 3-chloro-2-hydroxy isomer (LXXIb).

Scheme 19



4.2D. Reaction of trans-2,3-Epoxyoctadecan-1-ol (LXIII) with CTMS

Compound LXIII on reaction with CTMS afforded a TLC homogeneous solid product (LXXII, m.p.78-79C). Elemental analysis

corresponded to formula $C_{18}H_{37}O_2Cl$. IR spectrum showed absorptions at 3460 (OH), 1070, 1000 (C-O) and 810 (C-Cl). NMR spectrum gave the significant signals at τ 5.68-6.48 m (4H, $-\underline{CH}(Cl)-\underline{CH}(OH)-CH_2OH$) and 7.63 s (2H, 2 x $O\underline{H}$, exchangeable with D_2O). On the basis of mechanistic considerations and spectral data, the product LXXII was formulated as 2(3)-chloro-3(2)-hydroxyoctadecan-1-ol.

4.2E. Reaction of Methyl cis-12,13-epoxy-cis-9-octadecenoate (LXVIII) with CTMS

cis-Olefinic epoxy ester (LXVIII) when treated with CTMS in ether yielded an oily product LXXIII which showed a single spot on TLC. It was analyzed for $C_{19}H_{35}O_3Cl$ and responded to Beilstein test. Its IR spectrum showed bands at 3460 (OH), 3000 (=C-H str.), 1730 ($\underline{C}OOCH_3$) and 715 cm^{-1} (C-Cl). NMR spectrum exhibited signals at τ 4.58 m (2H, $-\underline{CH}=\underline{CH}-$), 6.05 m (1H, $-\underline{CH}(Cl)-$), 6.37 s (3H, $-CO_2\underline{CH_3}$), 6.5 m (1H, $-\underline{CH}(OH)-$, merged in part with the signal of methyl ester at τ 6.37), 7.62 s (1H, $O\underline{H}$, D_2O exchangeable) and 7.8 m (6H, $-\underline{CH_2}-CH=CH-\underline{CH_2}$ and $-\underline{CH_2}-CO_2CH_3$). On the basis of these data the product LXXIII was characterized as methyl 12(13)-chloro-13(12)-hydroxy-cis-9-octadecenoate.

It is important to mention here that the chlorohydrin formation takes place within minutes of the reaction and that too in a quantitative yield. In these reaction conditions, CTMS

does not effect the ester, hydroxyl and double bond groups. Thus chlorotrimethylsilane is a satisfactory reagent for the synthesis of chlorohydrins from epoxides without any side reaction.

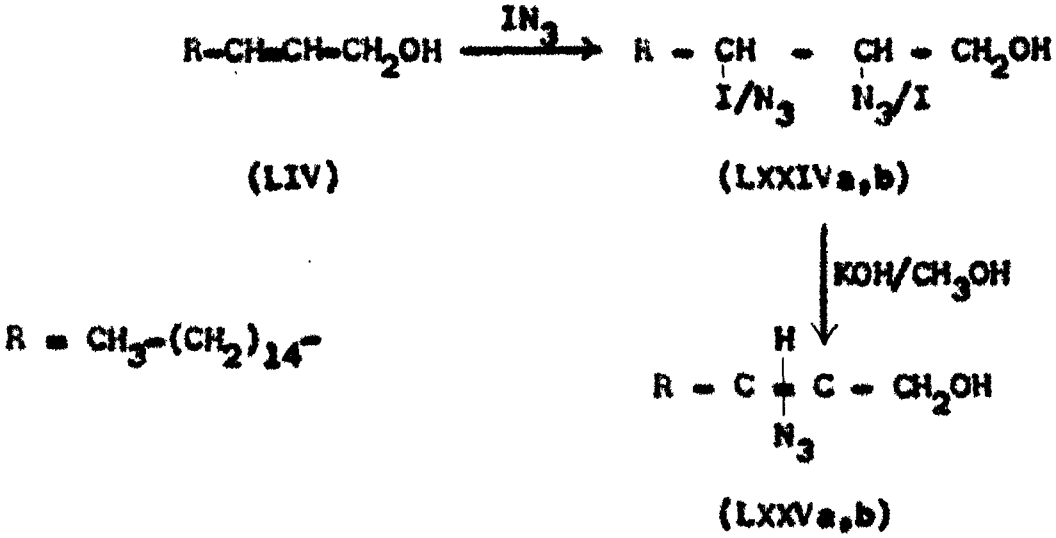
5. Reaction of Iodine-Azide with Long-Chain Allylic Alcohol

A variety of compounds of paramount importance can be synthesized from the iodoazide adduct, formed by the addition of iodine-azide (IN_3) to unsaturated compounds[76-78]. Foglia *et al.*[47] have investigated the additions of IN_3 to a few internal olefinic fatty esters. Addition of IN_3 to terminal, internal, α,β-unsaturated and acetylenic fatty acid esters were reported from author's laboratory[48,79]. Therefore, it was considered of interest to study this reaction with long-chain allylic alcohol. The resulting vinyl azides can be used for photolytic studies and are key intermediates in the synthesis of aziridines, azirines, triazoles and ketones[80]. Some of these compounds are of biological importance.

trans-2-Octadecen-1-ol (LIV) was treated with IN_3 according to the procedure of Fowler *et al.*[81] (Scheme 20). The addition product was found to occur in almost quantitative yield (LXXIVa,b ~ 97%). Evidence that addition indeed occurred was obtained from microanalysis and spectral data of the column purified adduct LXXIVa,b. Its elemental analysis corresponded to formula $C_{18}H_{36}ON_3I$ (positive Beilstein test). Its IR spectrum displayed a strong absorption at 2100 cm^{-1} attributable to the asymmetric stretching vibration of the azide functionality. Other bands were 3380-3260 (OH), 1070, 1050 (C-O) and 645 cm^{-1} (C-I). The NMR spectrum further confirmed the structure by

displaying signals at τ 5.92 m (1H, -C<u>H</u>-I), 6.46 m (2H,-C<u>H</u>$_2$-OH), 6.54 br,s (1H, O<u>H</u>, disappeared on addition of D_2O) and 6.68 m (1H, -C<u>H</u>-N_3).

<u>Scheme 20</u>



The mass spectrum of compound LXXIVa,b (Fig.9) gave conclusive support in favour of <u>erythro</u>-2(3)-azido-3(2)-iodo-octadecan-1-ol as an isomeric mixture. It gave molecular ion peak at m/z 437. The other prominent peaks were observed at m/z 420 (M-17), 409 (M-N_2), 395 (M-42), 351, 310 (M-127), 308, 282 (409-I or M-$C_{15}H_{31}$), 268 (282-14), 267 (M-167), 266 (M-171), 264, 254 (268-14), 250 (264-14), 240 (254-14), 238 (409-171), 226 (cleavage between C_3-C_4), 224 (266-42), 222 (254-32), 208, 198, 196, 171 (cleavage between C_2-C_3), 170, 168, 156, 154 (282-HI), 153 (152-H), 152, 138 (152-14), 128 (154-26), 127, 123, 86, 71, 58, 55 (base peak) and 41 (Chart 6).

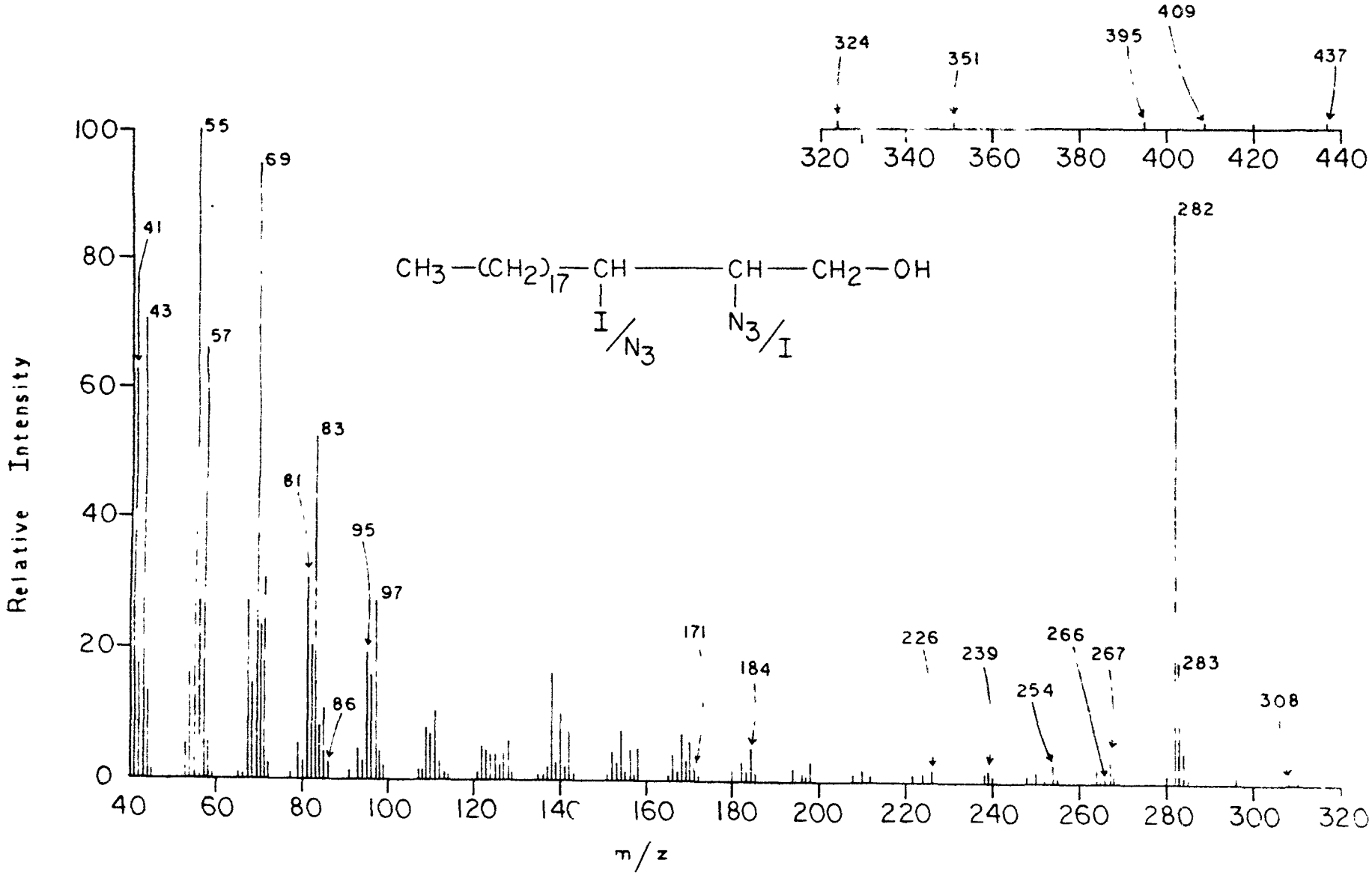


FIG 9 MS of LXXIVa,b

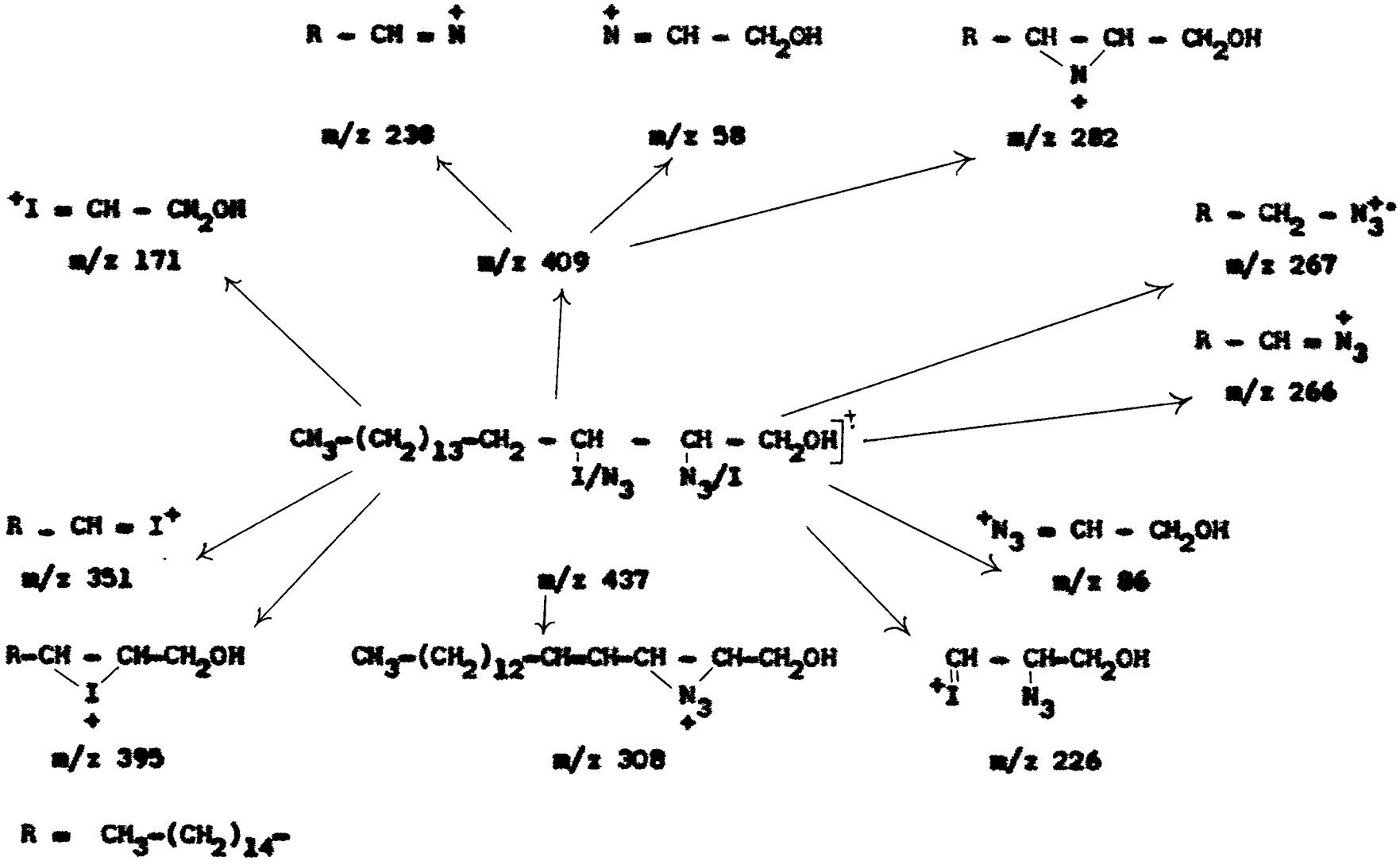


Chart 6. Mass Fragmentation of LXXIVa,b

The fragment ions m/z 266 and 86 of relatively low intensity showed that azide function is present at C_3 and C_2 position simultaneously. The fragment ion m/z 267 is further helpful in confirming the presence of isomers. Similarly the fragment ions m/z 351 and 171 established the presence of iodo-function at C_3 as well as C_2. These fragment ions clearly supported the fact that the product LXXIVa,b is an isomeric mixture.

Dehydroiodination of LXXIVa,b

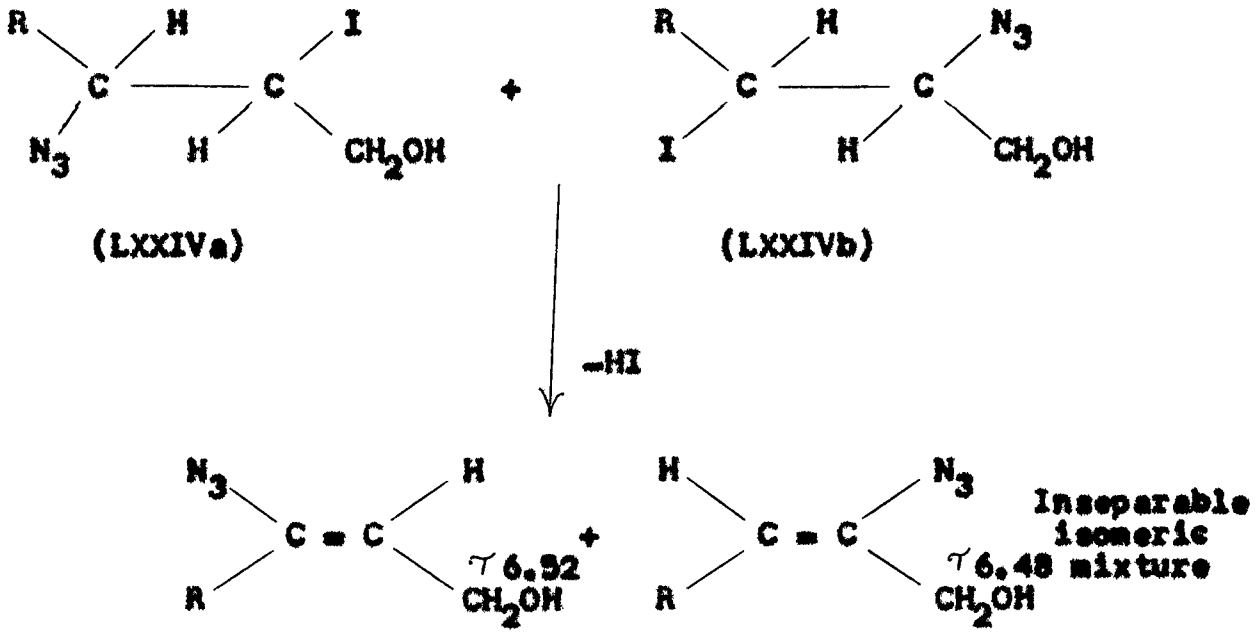
Dehydroiodination of LXXIVa,b with methanolic potassium hydroxide gave a quantitative yield of the corresponding cis-vinyl azide (LXXVa,b). Elemental analysis of LXXVa,b corresponded to the formula $C_{18}H_{35}N_3O$. These isomers did not respond to Beilstein test but showed unsaturation test. IR spectrum showed characteristic bands at 2100 and 3330 cm^{-1} assignable to the azide and hydroxyl functions, respectively. These bands are also present in iodoazide adduct (LXXIVa,b). The azide band at 2100 cm^{-1} is identical in position and intensity with that of iodoazide structure. However, a new absorption band located at 1650 cm^{-1} is attributed to stretching vibration of the carbon-carbon double bond in conjugation with the azide function.

Assignment of cis geometry to the vinyl azide derivative was made on the basis of known preference of E_2 elimination reaction to occur with a trans antiparallel arrangement of leaving group[81]. Verification of this assignment was made from the inspection of the NMR spectrum. The chemical shift of the vicinal proton which appeared in the area of τ 4.95 t (J=7 Hz)[81] is of particular importance. The absorption relatively at higher field (τ 4.95) for vinylic proton in LXXVa,b compared to normal vinylic protons can be attributed to the contributions from resonance structure (F) which increases the electron density at the vicinal carbon atom.

(E) (F)

The formation of isomeric cis-2(3)-azidooctadecen-1-ol (LXXVa,b) was based on the appearance of two typical absorptions; one at τ 6.48 s ($-CH = \underset{N_3}{C} - CH_2OH$] accounted for 76% of the total product and is attributed to cis-2-azidooctadecen-1-ol (LXXVb). The another absorption centered at τ 6.52 d ($-\underset{N_3}{C} = CH - CH_2OH$, J=6 Hz) accounted for remaining 24% as a cis-3-azidooctadecen-1-ol (LXXVa). Other NMR signals

were observed at τ 6.65 br,s (1H, OH, disappeared on D_2O shake), 7.95 m (2H, $-CH_2-C(H)(N_3)-C-CH_2OH$), 8.7 br,s (chain $-CH_2$) and 9.12 t (3H, CH_3-).



R = $CH_3-(CH_2)_{14}-$

Mass spectrometry was useful in confirming the structure of the vinyl azide. The mass spectrum of LXXVa,b (Fig.10) gave molecular ion peak at m/z 309. Other prominant peaks were observed at m/z 280 ($M-CH_3CH_2$), 266 (M-43 or 280-14), 264 (266-2H or 280-16), 252 (266-14), 250, 238 ($266-N_2$), 236 ($264-N_2$), 224 (238-14), 222 (236-14), 208 (222-14), 196(208-14),

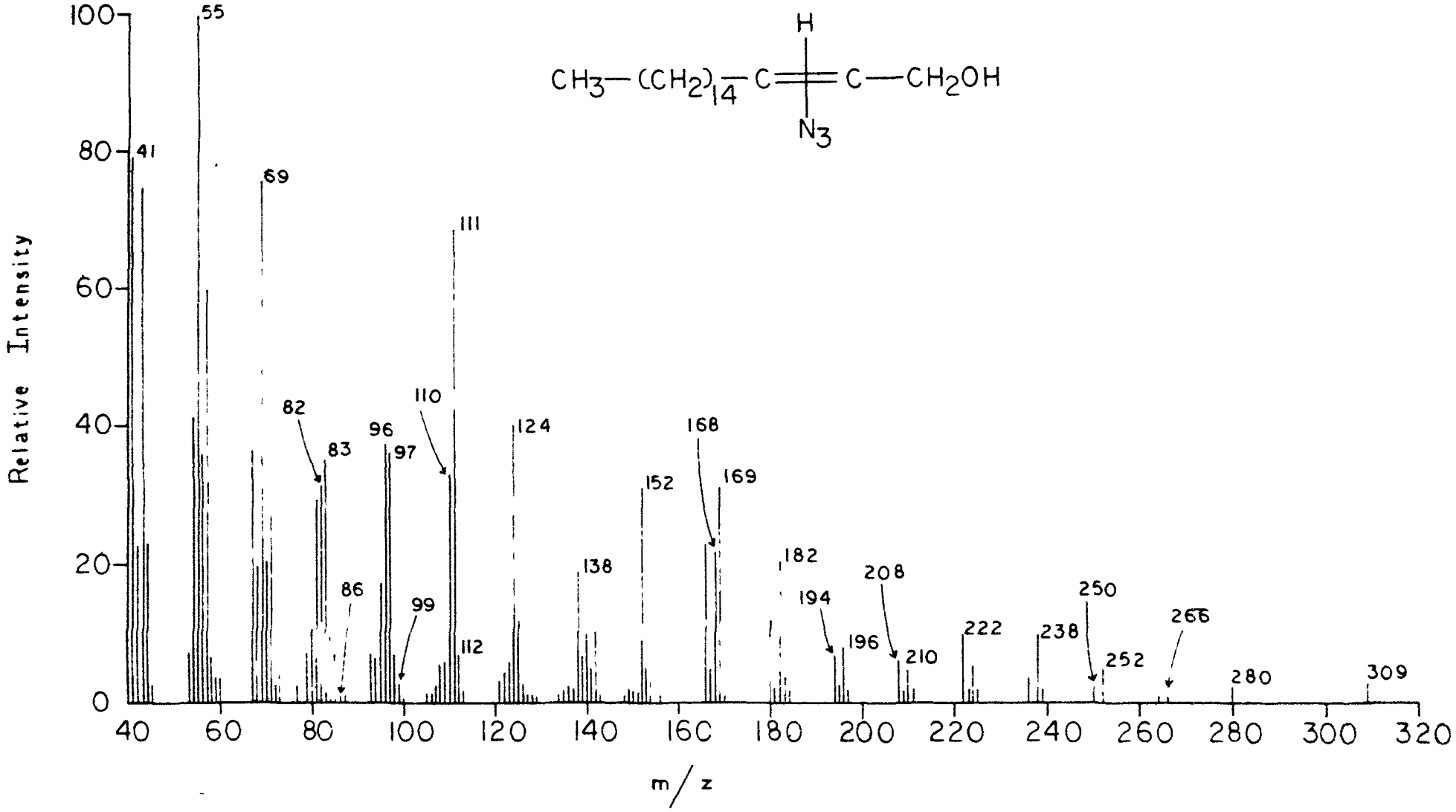


FIG. 10. MS of LXXVa,b

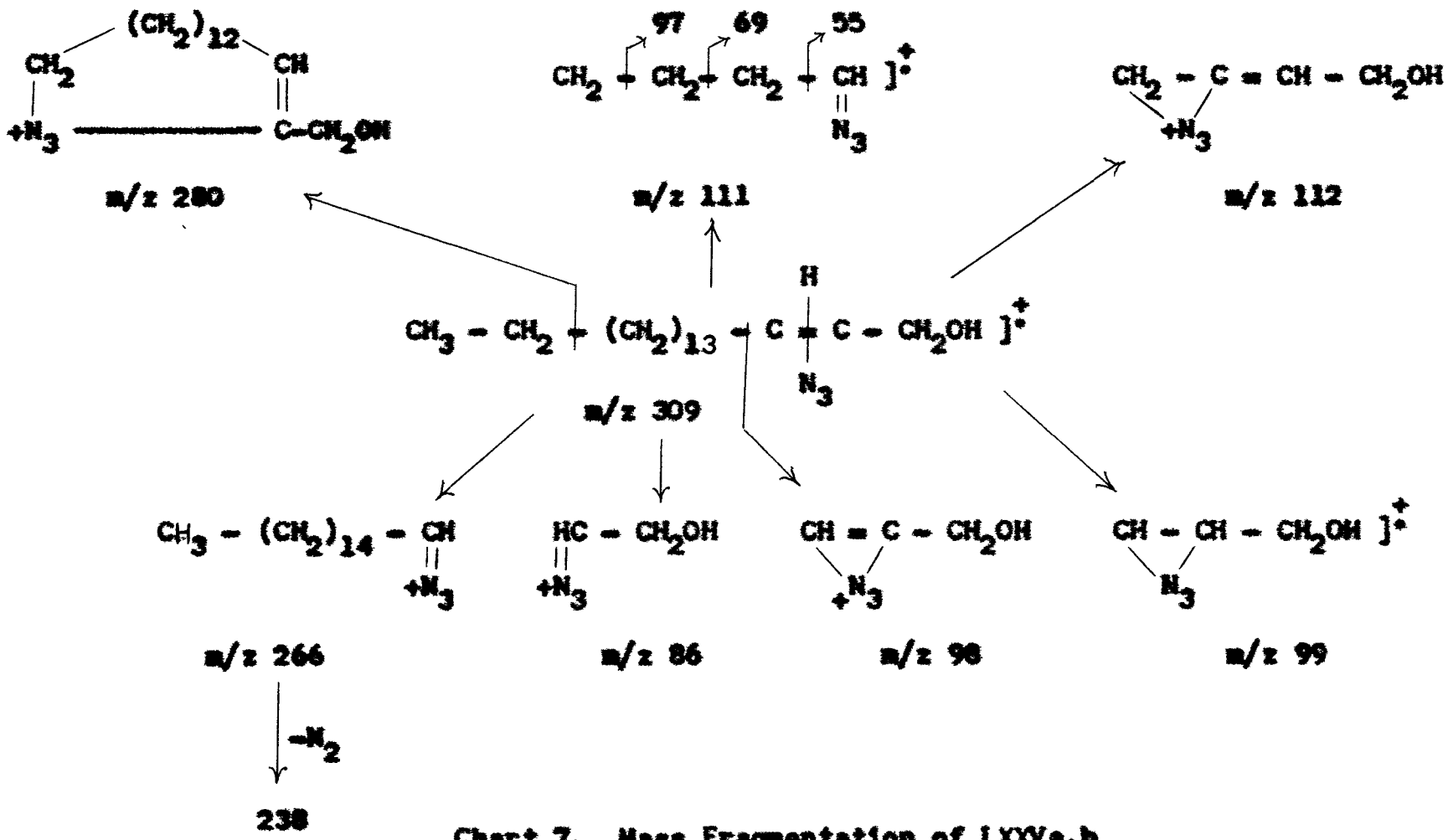


Chart 7. Mass Fragmentation of LXXVa,b

180 (196-14), 166, 152, 138, 124, 112, 111, 99 (M-210), 98 (M-211), 86, 85 (86-H), 71 (99-28), 70 (98-28), 69 (86-17), 68 (99-31 or 85-17), 67 (98-31), 55 (base peak) and 54 (68-14). Formation of some important fragment ions are shown in chart 7.

Fragment ions m/z 266, 112, 99, 98, 86 and 85 further supported the structure. An intense mass ion at m/z 238 observed by the loss of N_2 from m/z 266 suggested the incorporation of azido function at C_3, while ion at m/z 69 resulting by the loss of mass unit 17 from m/z 86 confirmed the presence of azido function at C_2 of the chain.

6. Preparation of Long-Chain 2,3-Aziridines

Aziridines are known to be in use as important pharmaceuticals, veterinary medicines, agrichemicals, antimicrobial and adrenoceptor blocking agents[82,83]. Preparation of 2,3-aziridines of fatty acids has received limited study. N-substituted 2,3-aziridine has only been reported[54] by the addition and then cyclization of pseudohalogen (NNDBS) to α,β-unsaturated fatty acid ester. Few reactions of ammonia with short-chain 2,3-dibromocarboxylic acid esters have appeared in the literature[57] for the synthesis of aziridines. Reaction of ammonia with long-chain methyl 2,3-dibromoester for the synthesis of aziridines has not been reported. As there is scanty information about fatty aziridines, it is considered of interest to develop a route for the preparation of 2,3-aziridines from methyl 2,3-dibromohexadecanoate (IIIb) using ammonia in methanol.

6.1. Bromination of Methyl trans-2-hexadecenoate (Ib)

Methyl 2,3-dibromohexadecanoate (IIIb, m.p.36-37C) was prepared from methyl trans-2-hexadecenoate (Ib) following the procedure of Myers[3]. The compound IIIb was analyzed for $C_{17}H_{32}O_2Br_2$ (positive Beilstein test). Its IR spectrum gave bands at 1740 ($\underline{C}OOCH_3$) and 650 cm^{-1} (C-Br). There was no evidence for the presence of an olefinic bond. The NMR spectrum

likewise was devoid of any signal for vinylic protons. It gave signals at τ 5.76 m (2H, $-\underline{CH}-\underline{CH}-CO_2CH_3$), 6.28 s (3H, $-CO_2\underline{CH_3}$), 8.78 br,s (chain $-\underline{CH_2}-$) and 9.13 t (3H, $\underline{CH_3}-$). In the light of the above informations IIIb was characterized as methyl 2,3-dibromohexadecanoate.

6.2. Reaction of IIIb with Ammonia

The reaction of IIIb with ammonia in methanol at OC yielded only one product LXXVI. It was analyzed for $C_{17}H_{31}O_2Br$. The IR spectrum gave bands at 1725, 1715 ($\underline{COO}CH_3$), 1610 (C=C), 1215, 1120, 1050 (C-O) and 645 cm^{-1} (C-Br). The NMR spectrum displayed a triplet centred at τ 3.39 (J=8 Hz) ascribable to a proton β to ester carbonyl. A multiplet centered at τ 7.5 was assigned to C_4 methylene protons. With the help of these data the compound LXXVI was characterized as methyl 2-brome-2-hexadeceneate.

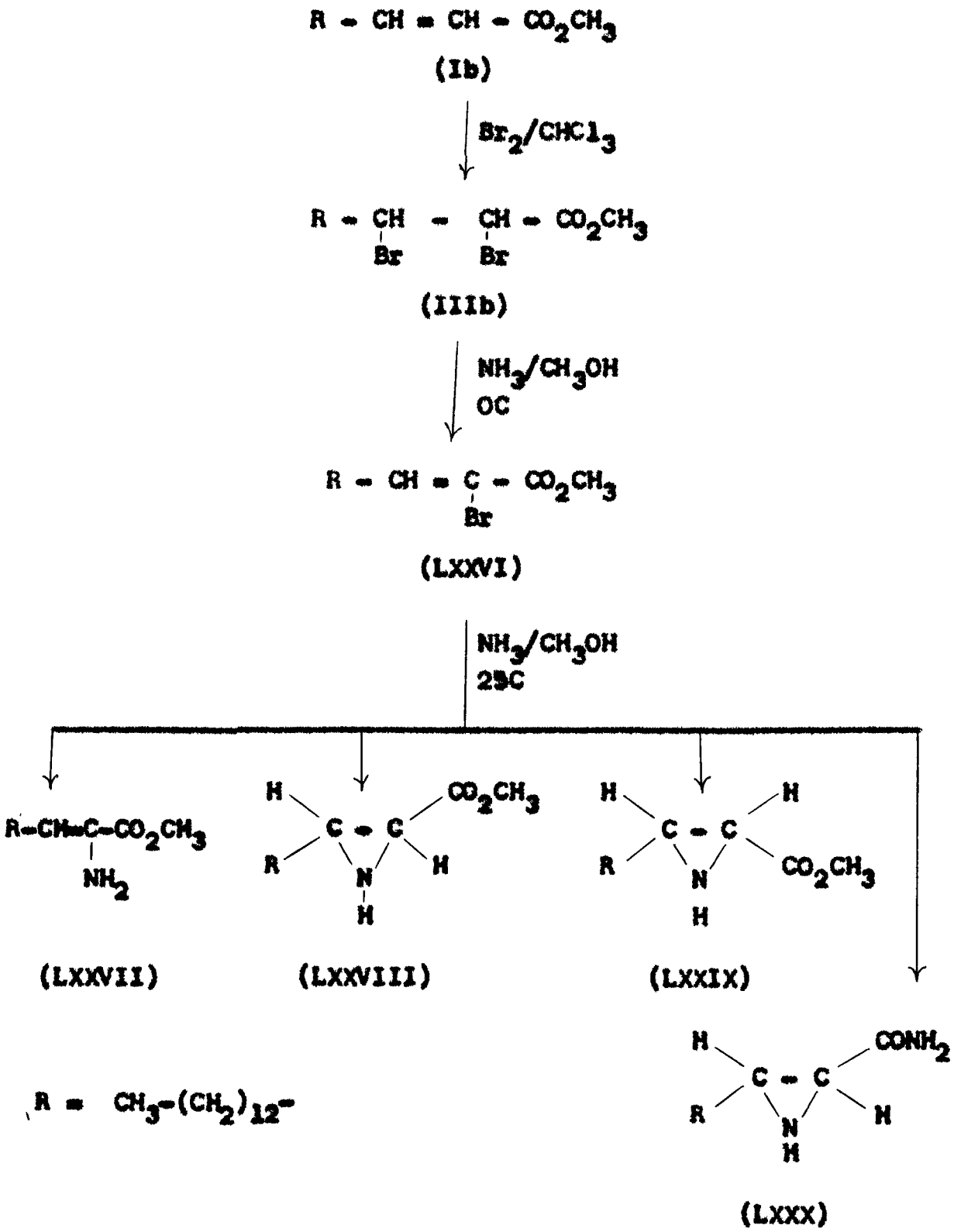
6.3. Reaction of LXXVI with Ammonia

Treatment of methyl 2-brome-2-hexadeceneate (LXXVI) with a solution of ammonia in methanol at 25C afforded four TLC homogeneous products; (LXXVII - LXXX) (Scheme 21).

Characterization of Product LXXVII

Elemental analysis of pure product corresponded to formula $C_{17}H_{33}NO_2$. The IR spectrum exhibited characteristic

Schema 21


R - CH = CH - CO2CH3
(Ib)
Br2/CHCl3
R - CH(Br) - CH(Br) - CO2CH3
(IIIb)
NH3/CH3OH
OC
R - CH = C(Br) - CO2CH3
(LXXVI)
NH3/CH3OH
25C
R-CH=C(NH2)-CO2CH3
(LXXVII)
(LXXVIII)
(LXXIX)
(LXXX)
R = CH3-(CH2)12-

band at 3430-3300 for NH_2 group. Two bands of almost equal intensities in carbonyl region at 1730 and 1720 were recorded. The splitting[84] of said carbonyl frequency occurs due to the presence of a substituent α to ester carbonyl. The intense band at 1620 cm^{-1} was ascribable to C=C stretching. A strong band at 1240 and the weak band at 1040 cm^{-1} characteristic of C-N stretching argued the presence of amino function attached with the tertiary α-carbon atom[85]. Other bands at 1130, 1030, 1010 and 1000 (C-O) were accompanied by an intense broad band at 750 cm^{-1} attributed to wagging and twisting vibration of NH_2 group.

NMR spectroscopy was much useful for the confirmation of the structure. The diagnostic signals were observed at τ 2.77 t (1H, $-\underline{CH}=\underset{|}{C}-CO_2CH_3$, J=7 Hz), 6.13 s (2H, $=\underset{|}{C}-\underline{NH_2}$), 6.21 s (3H, $-CO_2\underline{CH_3}$) and 7.75 m (2H, $-\underline{CH_2}-CH=\underset{|}{C}-$).

On the basis of microanalysis and spectral data compound LXXVII was identified as methyl 2-amino-2-hexadecenoate (α-dehydroamino ester, DHA). The literature has reports[86,87] that DHA is of great importance as a starting material for the incorporation and synthesis of dehydropeptides and peptide antibiotics.

Characterization of Product LXXVIII

Compound LXXVIII obtained as a white solid (m.p.39-40C), analyzed for $C_{17}H_{33}NO_2$. Its IR spectrum showed a weak

band at 3280 and the strong one at 855 cm^{-1}, the characteristic of trans-aziridine ring. It also showed the strong band at 1730 cm^{-1} for ester function, 1360 (C-N) and 1220, 1190, 1125, 1090, 1030 cm^{-1} (C-O). When the spectrum was recorded in $CHCl_3$, the characteristic absorptions of aziridine function shifted at 3270, 850 instead of 3280 and 855 cm^{-1}. These bands have been quoted to either deformation or vibration of the aziridine ring[88,89]. Its NMR spectrum displayed a diagnostic broad absorption at τ 7.84 (2H, -CH - CH-CO_2CH_3) along with a signal at 8.02 s (1H, >NH, D_2O exchangeable).

A combination of above evidences confirmed the structure of LXXVIII as methyl trans-2,3-epiminohexadecanoate.

Additional support in favour of the structure of LXXVIII was obtained by a study of its mass spectrum. The mass spectrum (Fig.11) marked molecular ion at m/z 283. The other important peaks were observed at m/z 284 (M+1), 268 (M-15), 254 (M-29), 252 (M-31), 240 (M-43), 226 (240-14), 225, 224 (M-59), 212 (226-14), 210 (M-73), 198 (212-14), 184 (198-14), 180, 170 (184-14), 156 (170-14), 142 (156-14), 129, 128 (base peak), 124, 115, 114 (115-H), 113 (M-170), 110, 102, 100, 97 (128-31), 96 (128-32), 88, 86, 87, 84 and 56 (84-28). The genesis of a few structure-revealing ions are suggested to occur according to scheme given below:

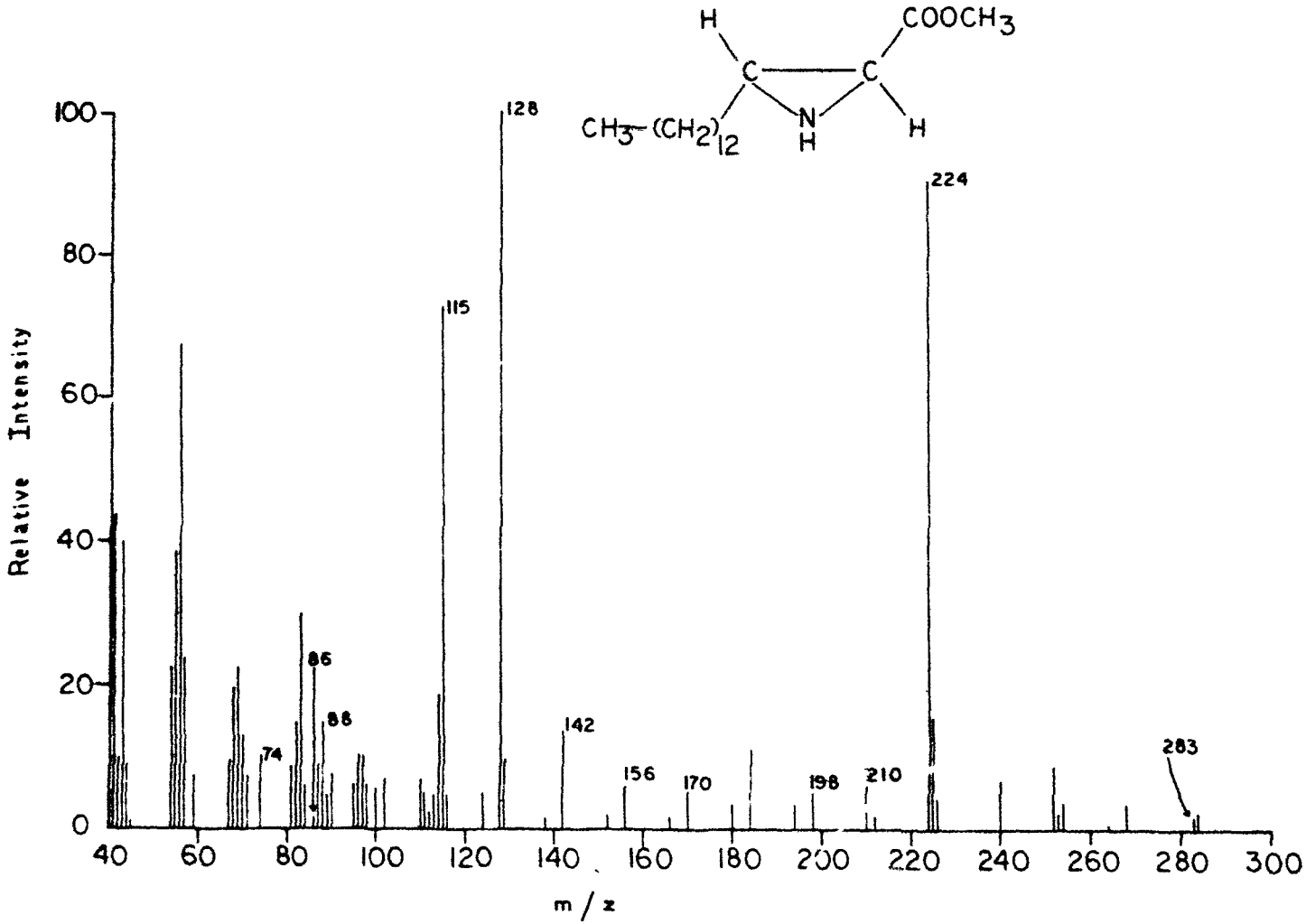
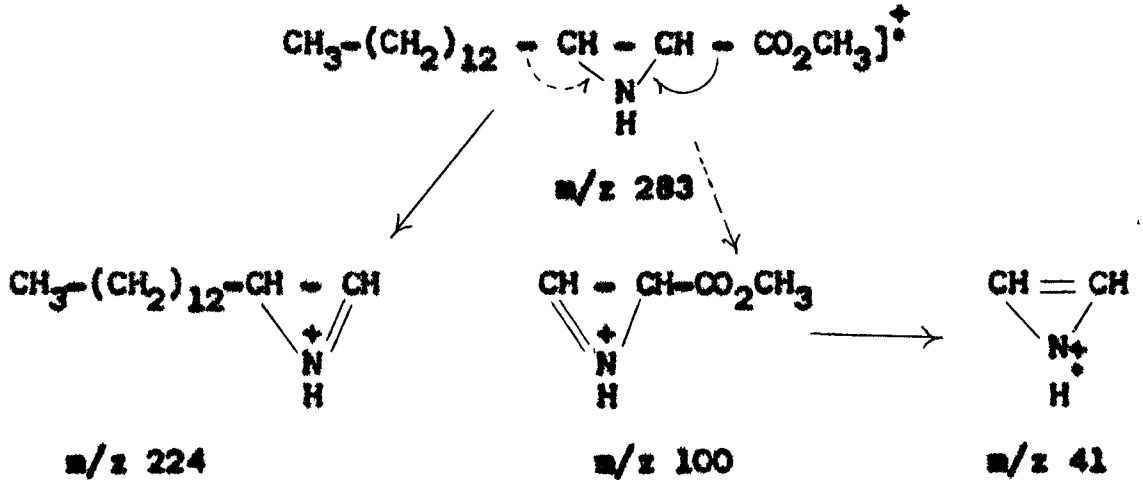


FIG.11. M S of LXXVIII

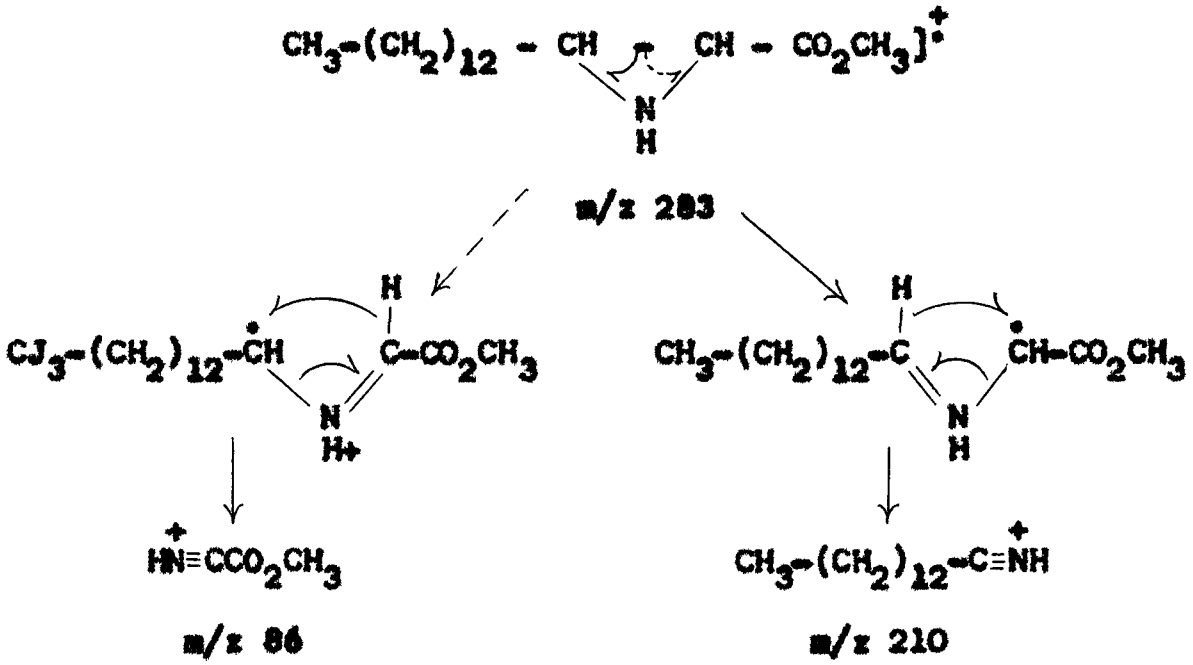
m/z 224 (M-59) and m/z 100 (M-183)

These two characteristic fragment ions M-59 and M-183 showed the presence of a ring at C_2-C_3 position. These can be shown to arise by the cleavages between C_1-C_2 and C_3-C_4, respectively. Fragment ion m/z 100 on further fragmentation gave ion at m/z 41.



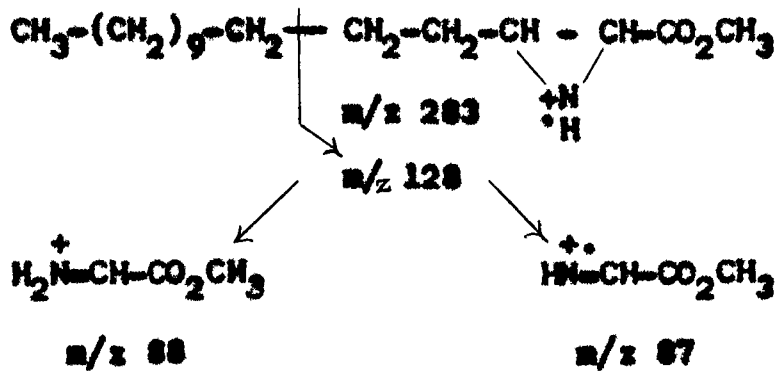
m/z 210 (M-73) and 86 (M-197)

These mass fragments originate from the molecular ion via transannular fragmentation with one hydrogen transfer. They are considered of significance to locate the position of ring at C_2-C_3.



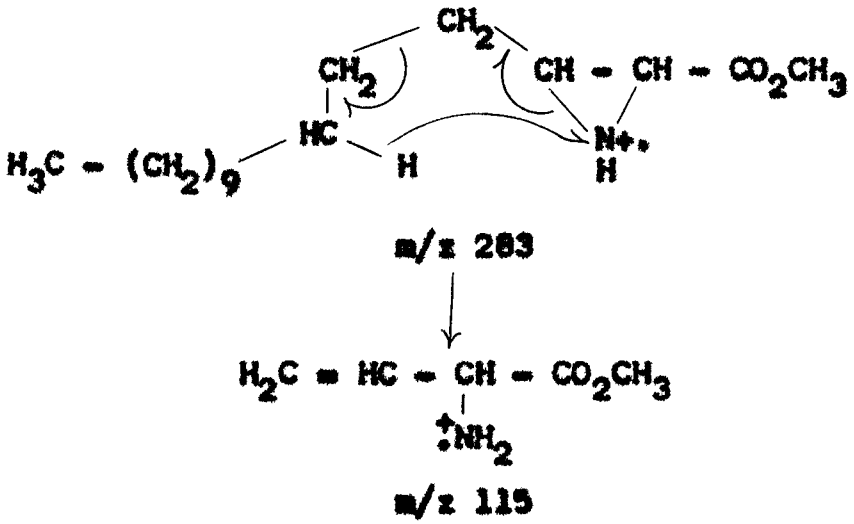
m/z 128, 88 and 87

The fragment ion m/z 128 constitutes the base peak of the spectrum and arises from γ-cleavage to ring. This ion further loses m/z 31, 32 and 59 mass units to give ions at m/z 97, 96 and 69, respectively. The rearranged fragments at m/z 88 and 87 from m/z 128 support much towards confirmation of the presence of aziridine ring at C_2-C_3.



m/z 115 (M-168)

This is a fairly strong peak and descends from the molecular ion peak via McLafferty rearrangement. The m/z 115 loses mass units 31 and 32 to give the fragment ions at m/z 84 and 83 respectively.



Characterization of Product LXXIX

The combustion data of compound LXXIX (m.p.56-57C) corresponded to formula $C_{17}H_{33}NO_2$. Its IR spectrum showed absorption bands at 3170 and 845 cm^{-1} for cis-aziridine ring. These values for aziridine ring are slightly different than those reported[98] for cis-9,10-epiminooctadecanoate (3150 and 840 cm^{-1}). The spectrum also displayed usual bands at 1735 ($\underline{CO}OCH_3$), 1410, 1370 (C-N) and 1180, 1135, 1015 cm^{-1} (C-O). As supporting evidence NMR spectrum exhibited characteristic

signals at τ 6.27 s (3H,-CO_2CH_3), 7.56 d (1H,-CH(N)-CH-CO_2CH_3, J=6 Hz), 7.91 m (1H,-CH(N)-CH-CO_2CH_3) and 8.21 s (1H, >NH, D_2O exchangeable). These data suggested the structure of compound LXXIX as methyl cis-2,3-epiminohexadecanoate, later confirmed by mass spectral study.

The mass spectrum (LXXIX, Fig.12) showed molecular ion peak at m/z 283 and base peak at m/z 128. Similar fragmentation pattern was observed as that of trans-aziridine (LXXVIII) with slight intensity differences.

Characterization of Product LXXX

The microanalysis of compound LXXX (m.p.116-117C) corresponded to formula $C_{16}H_{32}N_2O$. Its IR (KBr) spectrum exhibited characteristic bands at 3380, 3180 (NH_2), 3275, 855 (trans-aziridine ring) and 1305, 1165, 1130, 1040 cm^{-1} (C-O). A band at 3180 was probably an overtone of NH_2 deformation at 1620 cm^{-1}. The diagnostic amide absorption at 1650 (-$CO$$NH_2$) suggested the displacement of ester by amide group. In solution ($CHCl_3$) these bands moved to slightly higher or lower frequencies, viz: 3500, 3380 (NH_2), 3280, 850 (aziridine ring), 1675 (-$CO$$NH_2$) and 1585 cm^{-1} (NH_2 deformation, weak). A weak NH deformation was also noteworthy at 1560 cm^{-1} in solution. These differences result from hydrogen bonding[91]. Broad bands at 665

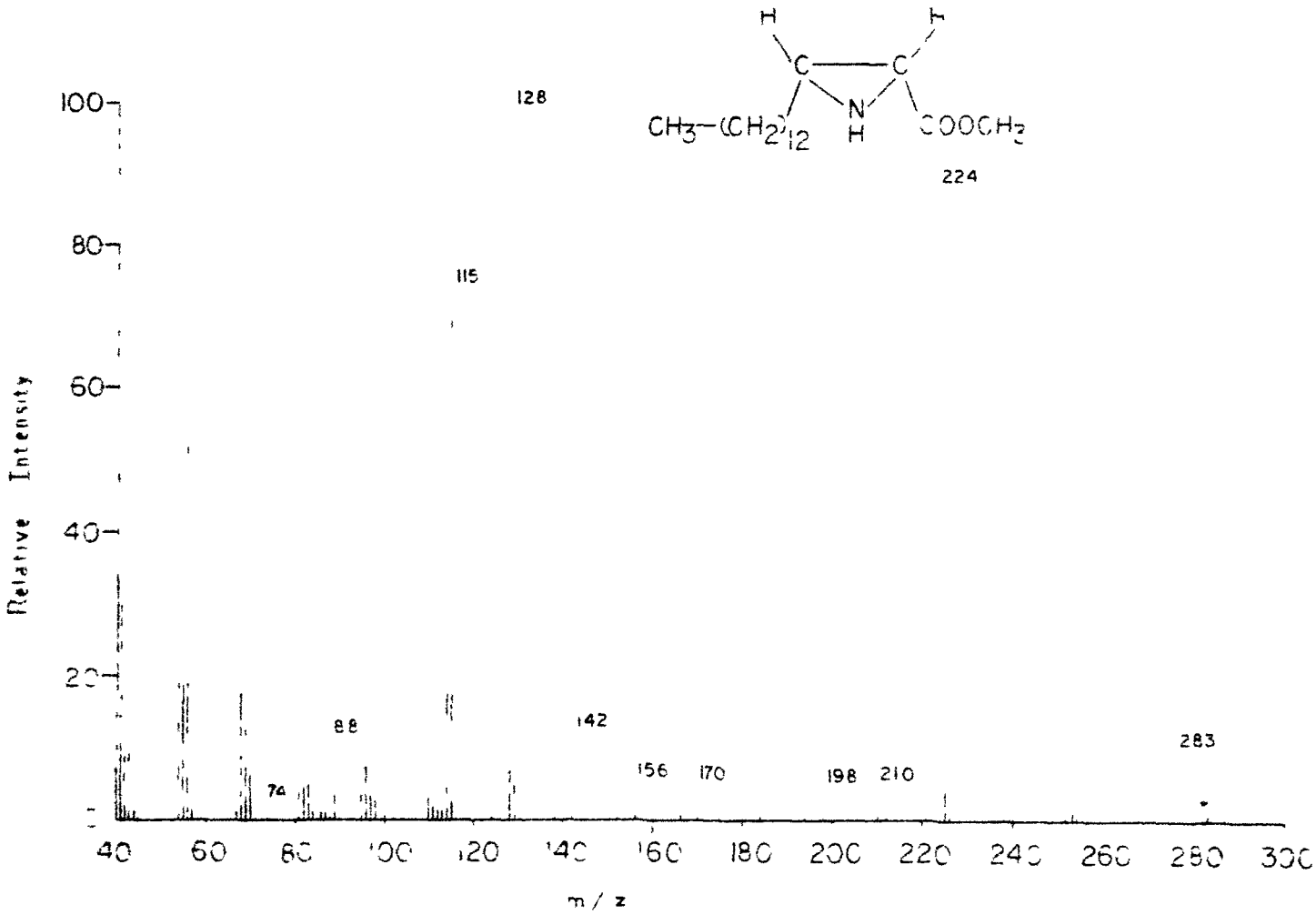


FIG 12 MS of LXXIX

and 625 cm^{-1} were assigned to NH_2 wag. (out of plane). Further confirmation of the structure of LXXXwas obtained from the mass spectral study.

The mass spectrum of LXXX (Fig.13) gives molecular ion peak at m/z 268 along with 269 (M+1). Other salient peaks are at m/z 252 (M-NH_2), 225, 224 (252-CO or M-44), 211 (M-57), 210 (M-58), 194 (210-NH_2), 180 (194-14), 169 (M-99), 155 (169-14), 141 (155-14), 127 (141-14), 113 (127-14), 110, 100 (M-168, base peak), 99 (113-14), 97 (113-16), 85, 83 (99-16), 73, 72, 71, 56 (100-44) and 55 (99-44). The genesis of some prominent structure-revealing ions are depicted in chart 8.

Fragment ions m/z 224 and 85 result from the α-cleavages with respect to aziridine ring. The mass ions at m/z 211, 210, 73, 72 and 71 originate via transannular cleavages with or without hydrogen transfer. The prominent ion at m/z 100 which constitutes the base peak is obtained by the usual McLafferty rearrangement. These mass ions confirm the position of aziridine ring at C_2-C_3. The other characteristic ions at m/z 99, 113, 127 and 141 are obtained by β, γ, δ and ϵ- cleavages, respectively. The loss of mass unit m/z 44 from the molecular ion shows the presence of -$CONH_2$ moiety in the molecule. These evidences established the structure of LXXX as <u>trans</u>-2,3-epiminohexadecamide.

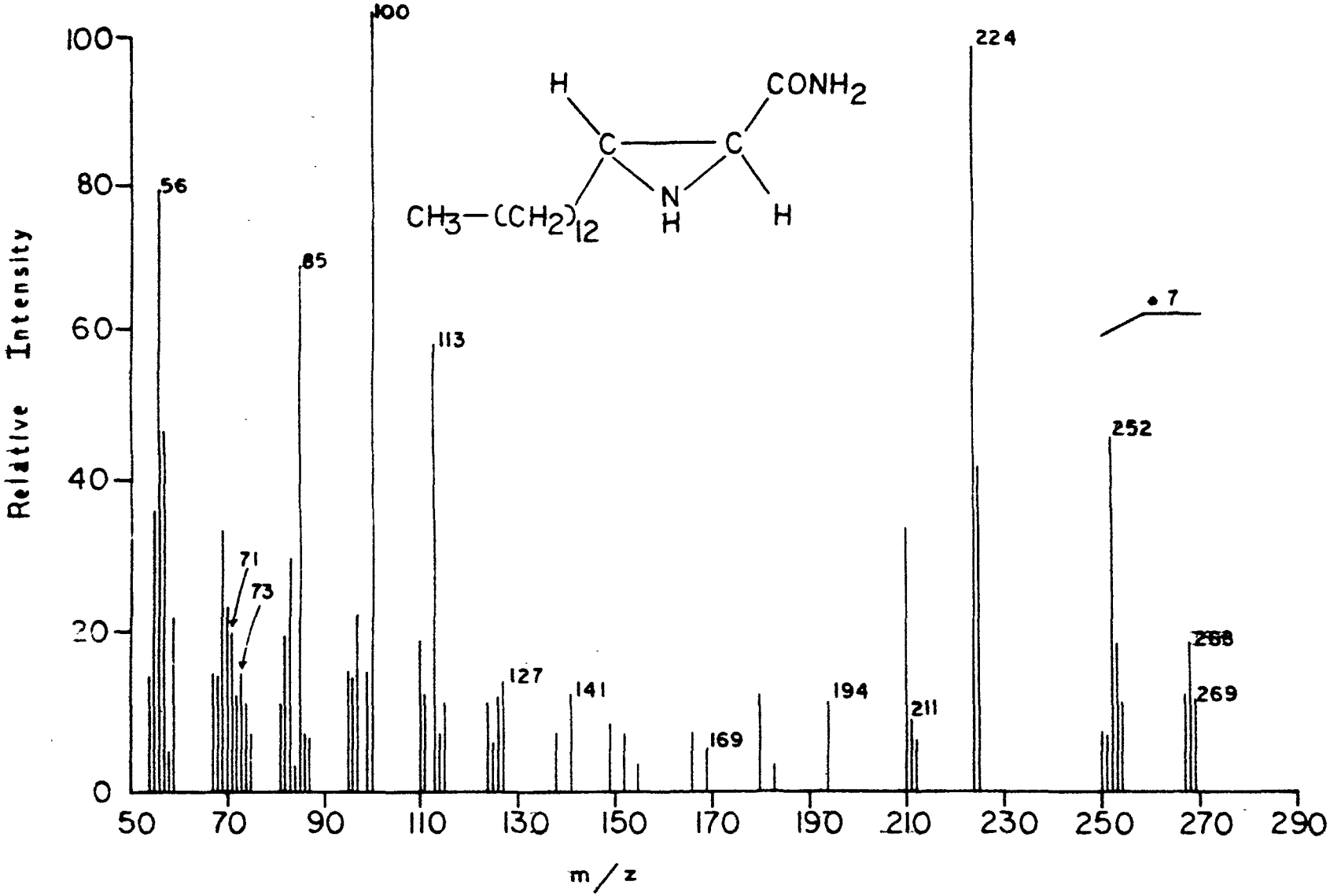


FIG. 13. M S of LXXX

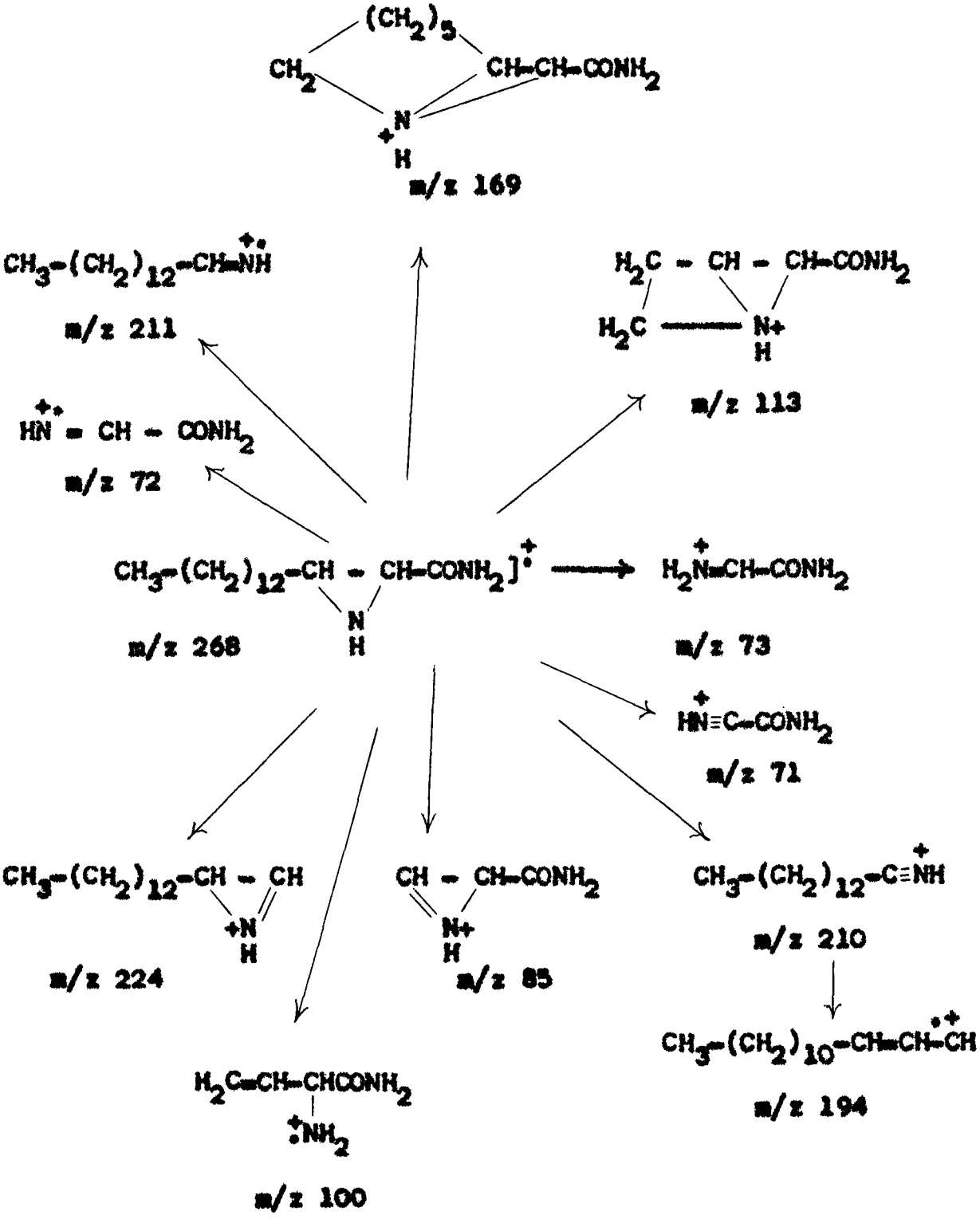
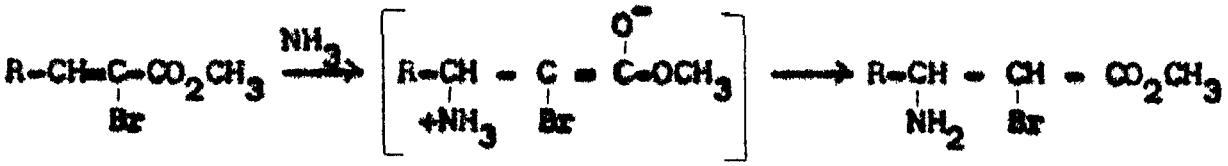


Chart 8. Mass Fragmentation of LXXX

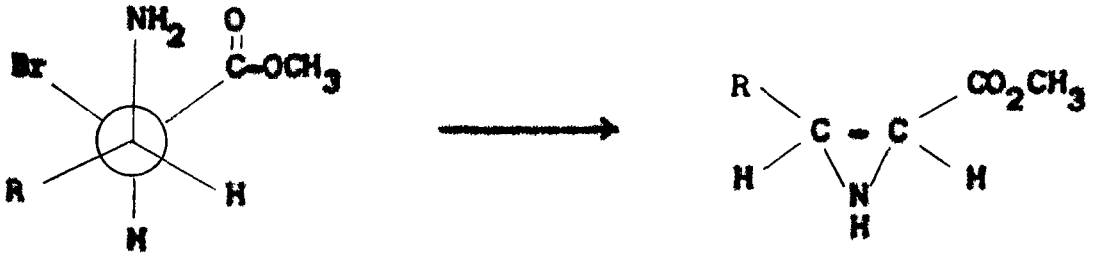
The probable mechanism for the formation of products LXXVIII and LXXIX is detailed in the scheme 22.

Scheme 22



(LXXVI) (LXXXIa,b)

Erythro (LXXXIa) trans (LXXVIII)



Three (LXXXIb) cis (LXXIX)

It is generally[38,92] agreed that the first step in the reaction of α,β-dibromocarbonyl compound with ammonia is dehydrohalogenation to α-bromo-α,β-unsaturated compound(LXXVI). This is then followed by 1,4-addition of the ammonia to produce

the α-bromo-β-amineester (LXXXIa,b). The composition of the mixture of erythro (LXXXIa) and three (LXXXIb) esters could not be determined since the components collapse immediately via intramolecular displacement of bromide to yield aziridines (LXXVIII and LXXIX). The possibility of the formation of amides of LXXVII and LXXIX in trace amounts defying detection and isolation can not be ruled out under the experimental conditions.

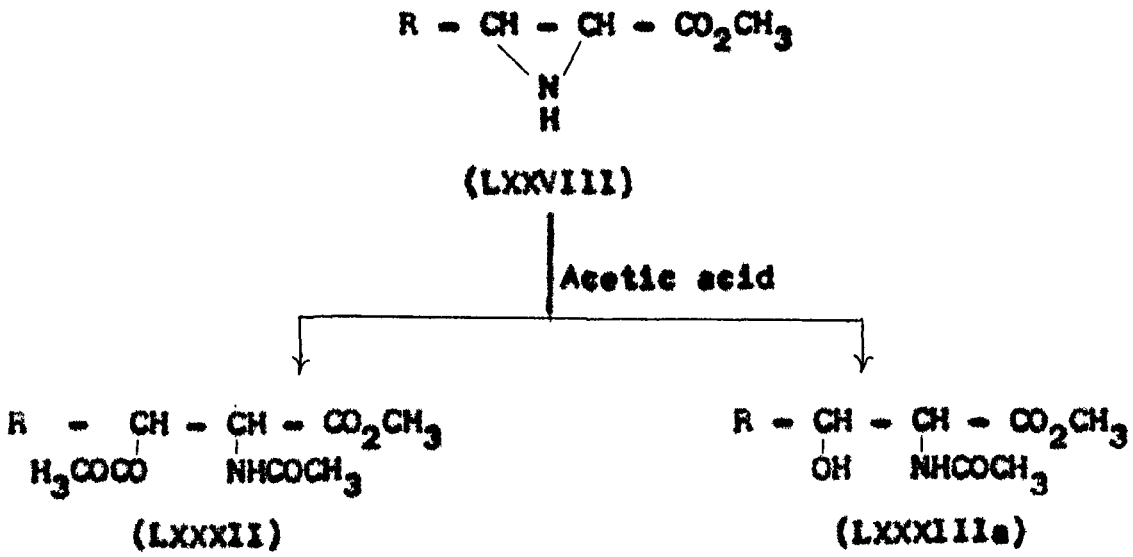
6.4. Reaction of Methyl trans 2,3-epiminohexadecanoate (LXXVIII) with Acetic Acid

Ring fission of compound LXXVIII with acetic acid was accomplished using the procedure of Maerker et al.[93] Usual workup by TLC monitoring and column separation yielded a semisolid product, LXXXII (~ 12%) followed by a major product LXXXIIIa (~ 88%) (Scheme 23).

Characterization of Product LXXXII

The microanalysis of compound LXXXII corresponded to formula $C_{21}H_{39}NO_5$. Its IR spectrum showed absorption bands at 3410 (NH str.), 1740 ($-O\underline{CO}CH_3$), 1730 ($-\underline{CO}OCH_3$), 1685 ($-NH\underline{CO}CH_3$) and 1265 (acetate). An intense band at 1370 was due to symmetric CH_3 deformation. The NMR spectrum gave the diagnostic bands at τ 5.80 m (1H, $-\underline{CH}-NHCOCH_3$), 6.15 m (1H,

Scheme 23



R = CH_3-$(CH_2)_{12}$-

-C<u>H</u>-OCOCH$_3$), 6.22 s (3H, -$CO_2C\underline{H}_3$), 7.95 s (3H, -OCOC<u>H</u>$_3$), 7.98 s (3H, -NHCOC<u>H</u>$_3$, merged in parts with signal of acetate protons at τ 7.95), 8.1 m (1H, >NH, slow D_2O exchangeable) and usual peaks as observed in fatty acid esters.

Further confirmation of its structure was obtained from the mass spectral study. The mass spectrum (LXXXII, Fig.14) gave molecular ion peak at m/z 385 along with 327 (M-58), 326 (M-59), 325 (M-60), 294 (326-32 or 325-31), 293 (325-32), 284 (326-42), 283 (326-43), 282 (326-44), 279 (294-15), 268 (326-58 or 327-59), 267 (326-59), 266 (284-18), 251 (279-28), 250 (294-44), 226 (268-42), 225 (268-43), 224 (283-59), 211 (283-72), 173, 171 (326-155), 157 (171-14), 149,

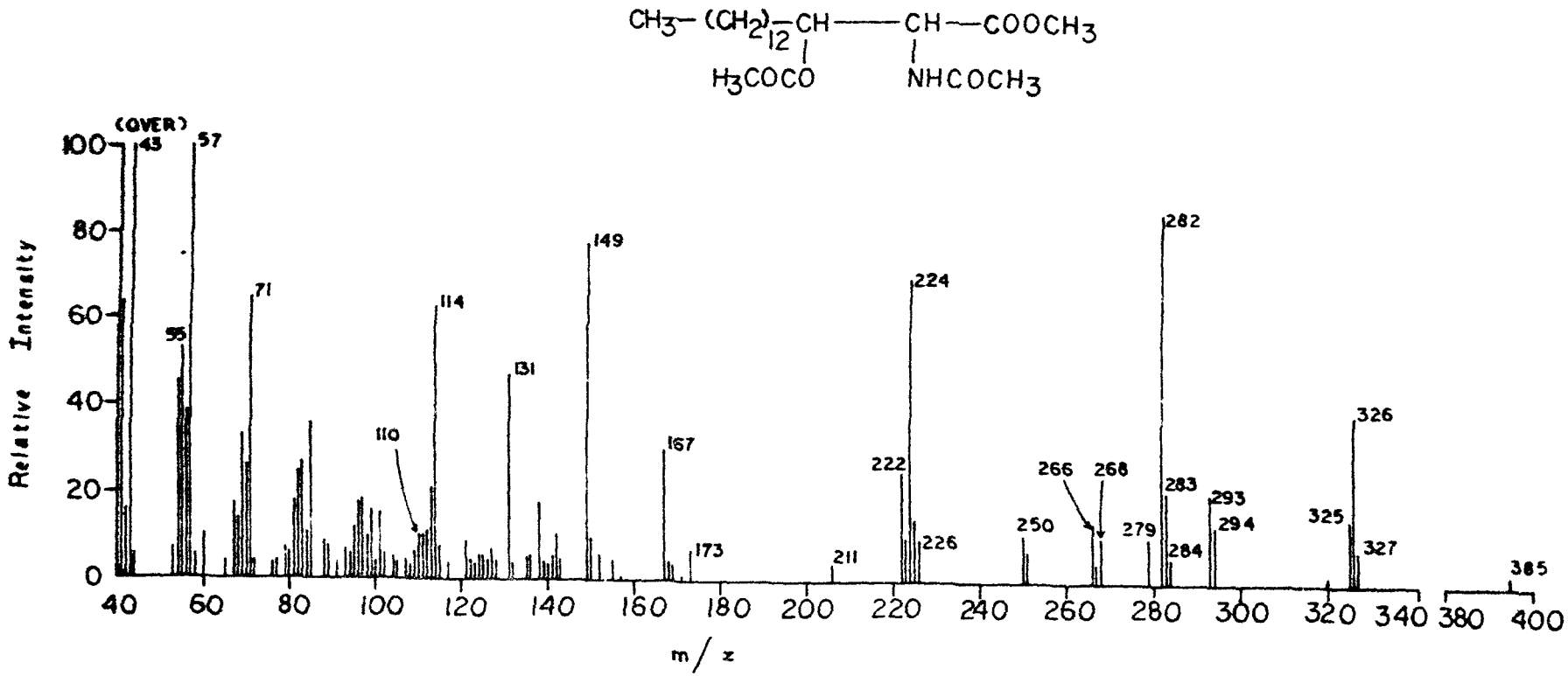


FIG. 14. M S of LXXXII

143 (157-14), 131 (M-254, McLafferty rearrangement), 114, 113 (131-18), 100 (131-31), 99 (131-32), 71 (114-43), 57 (base peak) and 43. Genesis of some structure supporting ions are shown in chart 9. The structure 2-acetamido-3-acetoxyhexadecanoate (LXXXII) is fully supported by the diagnostic peak at m/z 131.

Characterization of Product LXXXIIIa

Product LXXXIIIa (m.p. 84C) was analyzed for $C_{19}H_{37}NO_4$. It showed characteristic IR bands at 3520 (OH), 3440, 3335 (NH), 1735 (-COOCH$_3$), 1680 (-NHCOCH$_3$), 1620 (NH def., weak), 1490, 1460, 1435, 1370, 1260 (acetate), 1125 and 1070 cm^{-1}. The NMR spectrum showed characteristic bands at τ 4.3 m (1H, OH, disappeared readily after D_2O shake), 5.68 m (1H, -CH - CO_2CH_3), 6.19 s (3H, -CO_2CH_3), 6.2 m (1H, -CH-OH, merged in parts with ester protons), 7.97 s (3H, -NHCOCH$_3$) and 8.08 m (1H, >NH, slow D_2O exchange).

Mass spectrum of the product LXXXIIIa (Fig.15) showed characteristic peaks at m/z 343 ($M^{\cdot+}$), 344 (M+1), 312 (M-31), 285 (M-58), 284 (M-59), 282 (M-61), 266 (284-18), 255 (284-29), 254 (285-31), 242 (284-42), 241 (284-43), 240 (284-44), 226 (284-58), 224 (242-18), 213 (M-130 or 312-99), 212 (M-131 or 213-H), 160 (M-183), 131, 118 (160-42), 100 (131-31), 99 (131-32 or 312-213), 89 (131-42), 87 (118-31), 59 (131-72) and 43. Genesis of some significant ions are shown in chart 10.

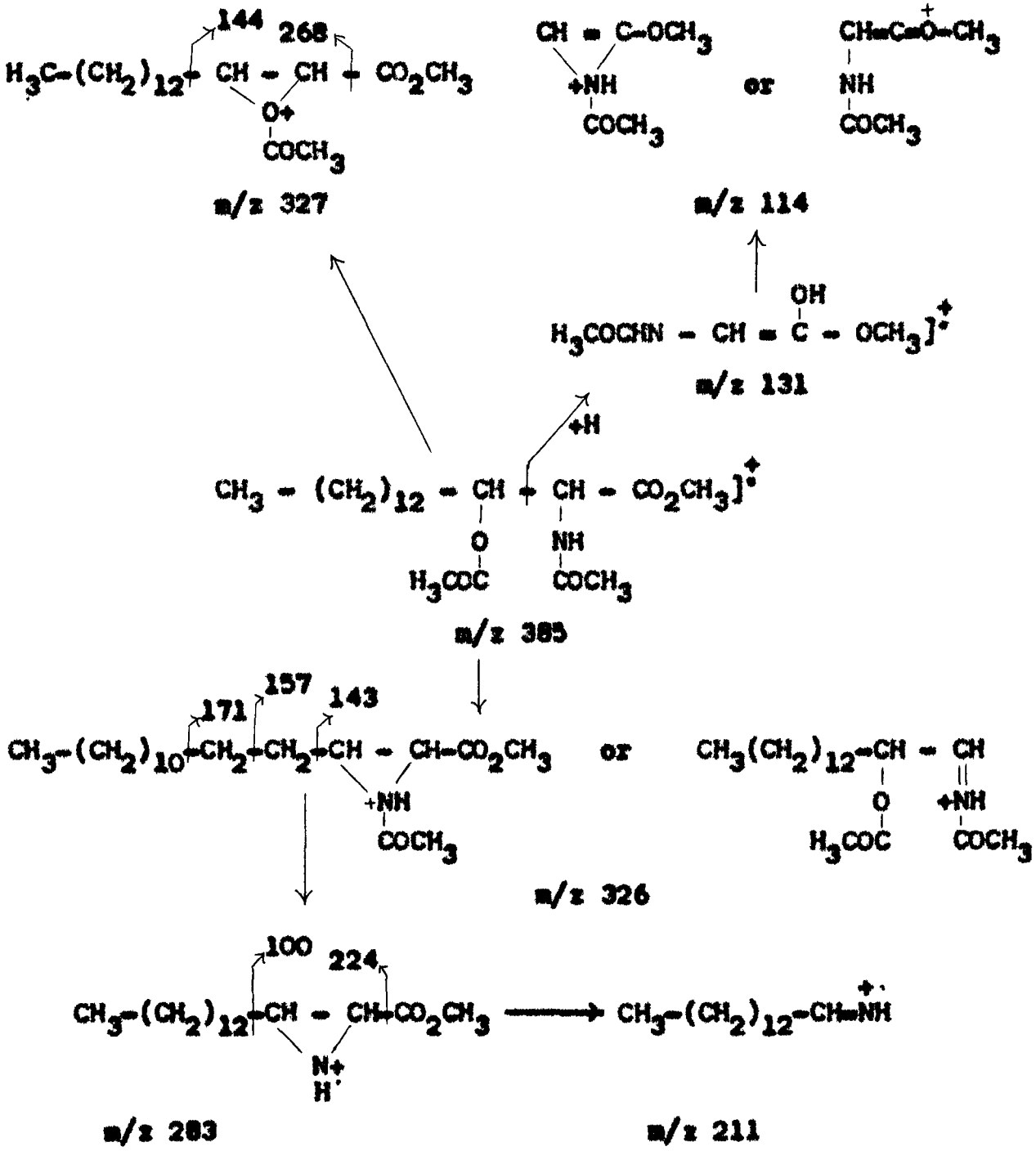


Chart 9. Mass Fragmentation of LXXXII

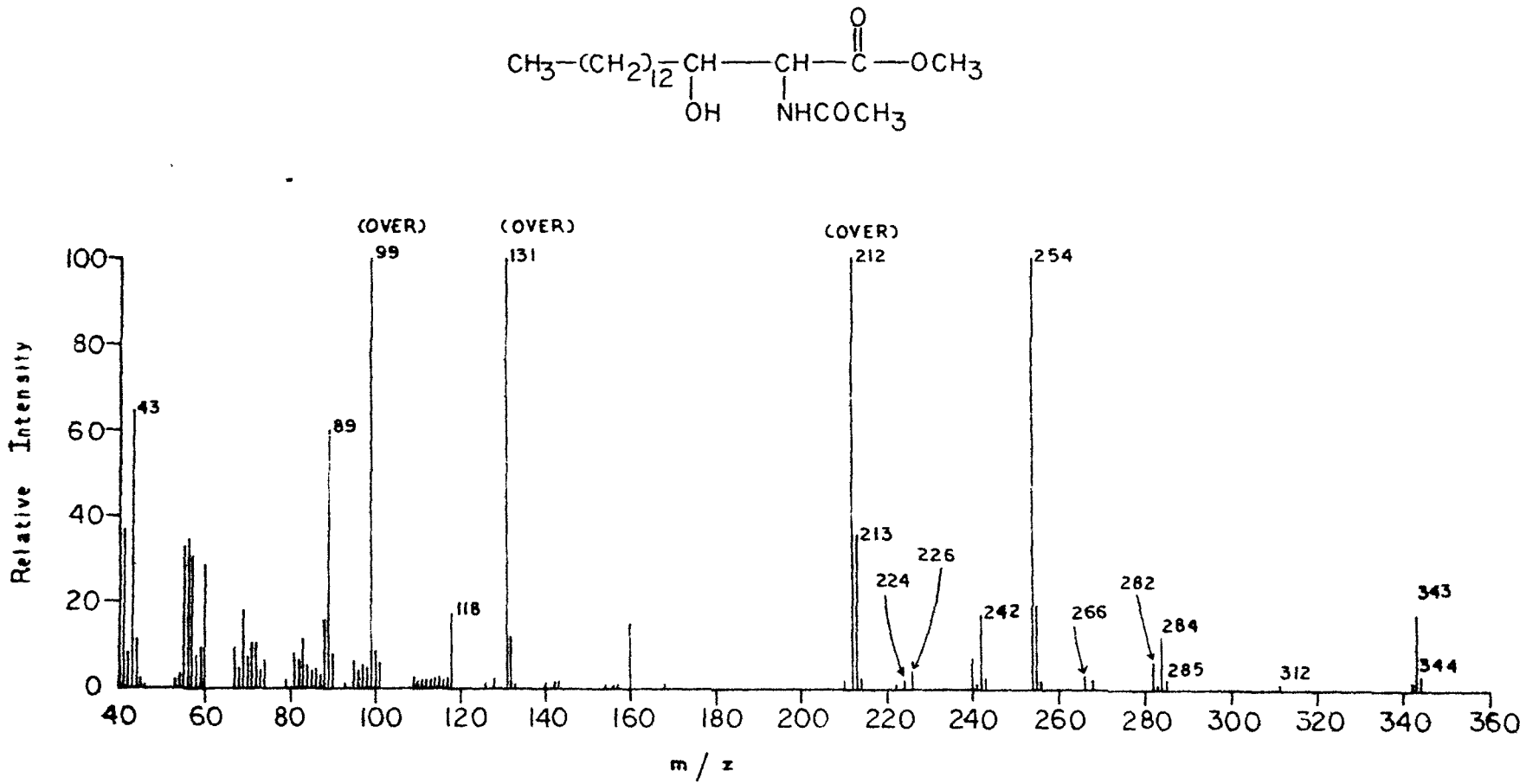


FIG. 15 MS of LXXXIIIa

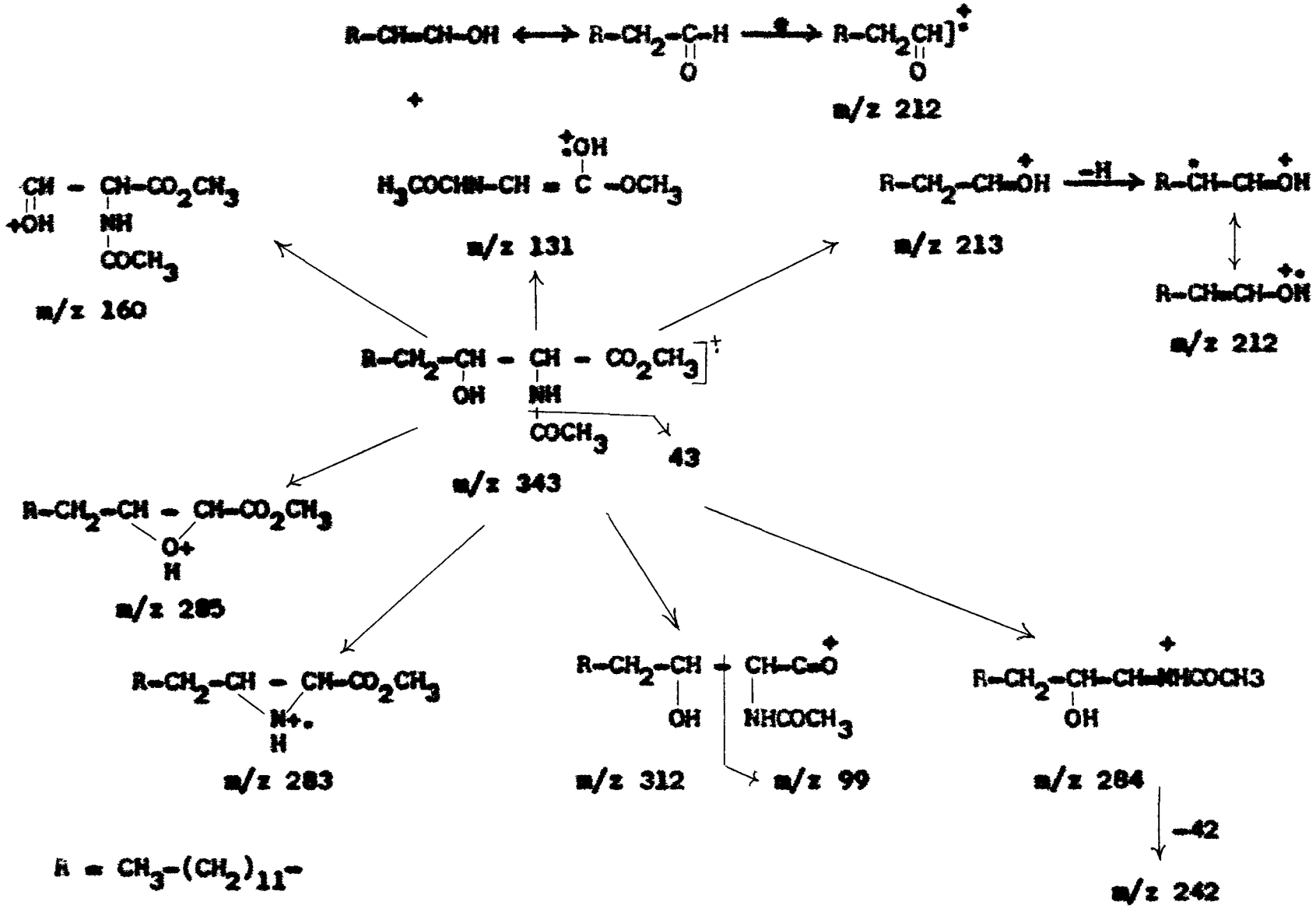


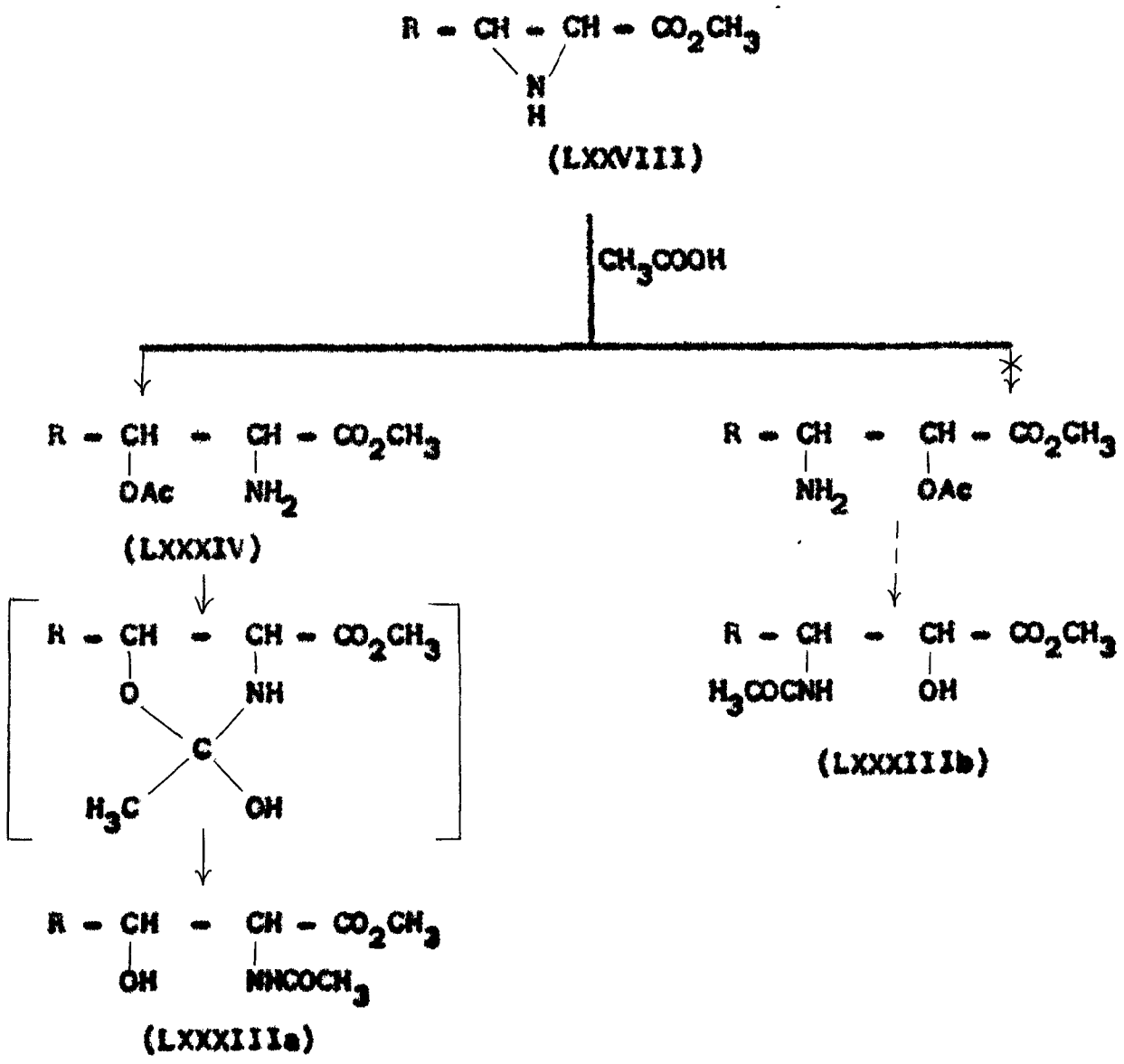
Chart 10. Mass Fragmentation of LXXXIIIa

The formation of fragment ion m/z 285 is due to loss of $NHCOCH_3$ from $M^{+\cdot}$. Further loss of OCH_3 from m/z 285 gives the base peak at m/z 254. The rupture of C_2-C_3 bond gives ion at m/z 213 which losses hydrogen leading to a strong peak at m/z 212. An intense peak at m/z 131 is a result of McLafferty rearrangement. The occurrence of the intense peaks (m/z 213, 212 and 131) confirms the attachment of acetamido and hydroxy groups to C_2 and C_3 respectively.

On the basis of above data the product LXXXIIIa was characterized as methyl-2-acetamido-3-hydroxyhexadecanoate.

The product LXXXIIIa is a rearranged product of LXXXIV. The latter appears to be formed as a result of initial protonation of the nitrogen atom followed by nucleophilic attack of acetate ion at C_3 of the aziridine ring (Scheme 24). The formation of β-hydroxyalkylamide from cis-2,3-dioctylaziridine with carboxylic acids has been reported[93]. Attempts to detect the compound LXXXIIIb failed thereby indicating the non-formation of the isomer or formation in trace amount.

Scheme 24


R - CH - CH - CO2CH3
N
H
(LXXVIII)
CH3COOH
R - CH - CH - CO2CH3
OAc NH2
(LXXXIV)
R - CH - CH - CO2CH3
O NH
C
H3C OH
R - CH - CH - CO2CH3
OH NHCOCH3
(LXXXIIIa)
R - CH - CH - CO2CH3
NH2 OAc
R - CH - CH - CO2CH3
H3COCNH OH
(LXXXIIIb)

Experimental

All melting points were observed on a Kofler apparatus and are uncorrected. Infrared (IR) spectra were determined using a Perkin-Elmer 621 and Pye Unicham SP-3-100 spectrophotometer. IR values are given in cm^{-1}. Ultraviolet (UV) spectra were recorded on a Perkin-Elmer 202 ultraviolet-visible spectrophotometer. Nuclear magnetic resonance (NMR) spectra were run on a Varian A60 instrument. Chemical shifts are reported as τ (ppm) relative to tetramethylsilane (TMS). ^{13}C NMR spectra were recorded on JEOL FX 90 model (90 MHz) instrument with proton noise decoupling. Mass spectra were measured with a JEOL JMS-D300 at 70 eV. In the absence of accurate and deuterated mass spectra the fragmentation pattern shown in discussion part may be considered as tentative. Thin layer chromatographic (TLC) plates were coated with silica gel G and sprayed with a 20% aqueous solution of perchloric acid. Petroleum ether (Pet. ether) refers to a fraction of b.p. 40-60C. The abbreviations 's, d, t, m and br' denote 'singlet, doublet, triplet, multiplet and broad', respectively.

Preparation of trans-2-Enoic Acids (Ia, XIa)

The trans-2-hexa-(Ia) and octadecenoic (XIa) acids were prepared from palmitic (XLVI) and stearic (XLVII) acids following the method of Palameta and Prostenik[7] as adopted as the standard procedure[10] in the authors' laboratory.

General Procedure

To a well stirred mixture of saturated acid (50 g) and red phosphorous (2.3 g), dry bromine (25 mL) was added dropwise at 90C in a period of 7 hr. The mixture was vigorously

stirred during the addition of bromine by using a mercury-sealed stirrer. Heating was continued for 24 hr. and the cooled solution was poured into cold water and left overnight. The product taken up in ether, washed successively with 10% aqueous sodium sulphite and distilled water and dried over sodium sulphate (Na_2SO_4). The 2-bromoacid obtained after evaporation of the ether was refluxed with powdered potassium iodide (48 g) in 95% ethanol (350 ml.) for 6 hr. To the cooled solution potassium hydroxide (32 g) was added and the mixture was refluxed for another 4 hr. Most of the alcohol was removed under reduced pressure and residue diluted with water, acidified with dilute hydrochloric acid and extracted with ether. The combined ether extracts were washed with water and dried. After evaporation of the solvent, a mixture of α,β-unsaturated and their co-products, i.e. 2-hydroxy and 2-ethoxy acids were obtained.

The 2-hydroxy acids (L, LI) were separated from α,β-unsaturated acids as copper chelate by treatment with cupric acetate in ethanol and acetic acid. The remaining two components obtained after removal of 2-hydroxy acids were fractionated by silica gel (BDH, 60-120 mesh) column chromatography to afford the individual components. Pure α,β-unsaturated acid was isolated by elution with pet. ether-ether (95:5, v/v) as a colourless product (yield ~32%), crystallized in pet.ether-ethanol (75:25, v/v). The 2-enoic acids on acid catalyzed esterification yielded their corresponding esters.

trans-2-Hexadecenoic acid (Ia, m.p. 54C, Lit. m.p. 53.5C)[94]. Analysis: Calcd. for $C_{16}H_{30}O_2$: C, 75.53; H, 11.89; Found: C, 75.47; H, 11.85%. (Ib) IR(CCl_4): 1730 ($\underline{C}OOCH_3$), 1650 (-CH=CH-) and 980 (trans-unsaturation). NMR(CCl_4): 3.1 d,d (1H, $-C\underline{H}=CH-CO_2CH_3$, J=15 and 5 Hz), 4.0 d (1H, $-CH=C\underline{H}-CO_2CH_3$, J=15 Hz), 6.29 s (3H, $-CO_2C\underline{H}_3$), 7.58 (m, 2H), 8.7 (br, s, 22H) and 9.14 (t, 3H).

trans-2-Octadecenoic acid (XIa, m.p. 58-59C, Lit. m.p. 58.5C)[94]. Analysis: Calcd. for $C_{18}H_{34}O_2$: C, 76.54; H, 12.13; Found: C, 76.52; H, 12.9%. The IR and NMR spectral data of XIb are same as those of Ib.

Preparation of Methyl 4-hydroxy-trans-2-hexadecenoate[16] (IVb)

NBS-Bromination of Methyl trans-2-hexadecenoate (Ib)

To the solution of Ib (5.0 g, 0.018 mol) in 100 mL anhydrous CCl_4 (dried over P_2O_5) crystallized NBS (3.31 g, 2.0 mol) and benzoyl peroxide (0.08 g) were added and the mixture was refluxed for 3 hr. After 3 hr. most of the NBS was converted to succinimide. The insoluble succinimide was filtered off, washed with CCl_4 and the bulk of the solvent was removed under reduced pressure. The residue taken in water was extracted with ether. Evaporation of the solvent gave a dark brown liquid ($\sim$ 6.5 g) which showed three distinct spots on TLC. The reaction product was purified by column chromato-

graphy using neutral alumina and a mixture of pet. ether-ether as eluting solvent. Elution with pet. ether gave the starting ester (Ib, 0.65 g, ~10%). Elution with pet. ether-ether (98:2, v/v) gave IIb (5.2 g, 80%).

Analysis: Calcd. for $C_{17}H_{31}O_2Br$: C, 58.78; H, 8.93; Br, 23.07; Found: C, 58.70; H, 8.81; Br, 22.85%. IR(CCl_4): 1730 ($\underline{COO}CH_3$), 1640 (-HC=CH-), 967 (trans-unsaturation) and 720 (C-Br). NMR (CCl_4): 3.8 d,d (1H, -$\underline{CH}$=CH-CO_2CH_3, J=15 Hz and 5 Hz), 4.8 d (1H, -CH=$\underline{CH}$-CO_2CH_3, J=12 Hz) and 5.6 m (1H, -$\underline{CH}$-Br), 6.2 (s, 3H), 8.7 (br, s, 22H) and 9.1 (t, 3H).

Elution with pet. ether-ether (95:5, v/v) gave methyl 2,3-dibromohexadecanoate (IIIb, 0.65 g, 10%), m.p. 35-36C, undepressed with an authentic specimen[16]. Ce-chromatography on TLC showed a single spot.

Alkaline Hydrolysis of Methyl 4-bromo-trans-2-hexadecenoate(IIb)

Compound IIb (3.5 g, 0.01 mol) was hydrolyzed by heating with KOH solution (2.5 g in 45 mL of ethanol-water in the ratio of 1:1) under reflux for 4 hr. The bulk of the solvent was removed under reduced pressure and the residue was diluted with ice-cooled water, acidified with HCl and extracted with ether. The yellow product (2.9 g) obtained after evaporation of the ether showed two distinct spots on TLC and was chromatographed over a column of silica gel (65 g). Elution

with a mixture of pet. ether-ether (80:20, v/v) followed by crystallization from pet. ether gave 4-oxo-hexadecanoic acid (Va, 0.83 g, ~30%, m.p. 95-96C).

Subsequent elution with pet. ether-ether (70:30, v/v) followed by crystallization with pet. ether gave IVa (1.91 g, ~70%, m.p. 70-71C). Its methyl ester was prepared with ethereal diazomethane which on crystallization from pet. ether afforded shining crystals of methyl 4-hydroxy-trans-2-hexadecenoate (IVb, m.p. 56-57C).
Analysis: Calcd. for $C_{17}H_{32}O_3$: C, 71.78; H, 11.34; Found: C, 71.65; H, 11.10%. IR(CCl_4): 3350-3270 (OH), 1730 ($-\underline{C}OOCH_3$), 1660 (-CH=CH-), 1170, 1140, 1080, 1050 (C-O) and 980 (trans-unsaturation). NMR($CDCl_3$): 3.1 d,d (1H, $-C\underline{H}=CH-CO_2CH_3$, J=15 Hz and 5 Hz), 4.0 d (1H, $-CH=C\underline{H}-CO_2CH_3$, J=15 Hz), 5.8 br (1H, $-C\underline{H}-OH$), 7.6 br (1H, $-O\underline{H}$, D_2O exchangeable), 6.24 (s, 3H), 8.74 (br, s, 22H) and 9.12 (t, 3H).

Preparation of trans-2-Octadecen-1-ol[62] (LIV)

Methyl trans-2-octadecenoate (XIb) (5 g, 0.017 mol) was dissolved in ether (10 mL) and gradually (5 minutes) added to a stirred solution of lithium aluminium hydride (LAH) (1 g) in dry ether (25 mL) at room temperature. Stirring was continued for additional 10 minutes. Excess of LAH was then destroyed with a mixture of ether-ethyl acetate (95:5, v/v). After acidification with cold 10% hydrochloric acid and usual

workup, the crude product (4.75 g) was chromatographed over a column of silica gel (60 g). Elution with pet. ether gave XIb (0.2 g, ~4.0%). Further elution with a mixture of pet. ether-ether (92:8, v/v) followed by crystallization from pet. ether gave trans-2-octadecen-1-ol (LIV, 4.4 g, ~92%, m.p. 46-47C, Lit. m.p. 45-48C)[63].

Analysis: Calcd. for $C_{18}H_{36}O$: C, 80.52; H, 13.52; Found: C, 80.50; H, 13.49%. IR(CCl_4): 3280 (OH) and 950 (-HC=CH-), 1040 (C-O). NMR(CCl_4): 4.7 m (2H, -CH=CH-), 6.43 unresolved doublet (2H, $-CH_2-OH$), 6.34 br,s (1H, -OH, D_2O exchangeable) and 8.04 m (2H, $-CH_2-CH=CH-$), 8.7 (br,s,26H) and 9.12 (t, 3H).

Preparation of Methyl 4-oxo-trans-2-hexa-(VI) and octadecenoate (LV)

A solution of chromium trioxide was prepared by addition of chromium trioxide (5 g, 0.05 mol) in small portion to a mixture of acetic anhydride (12.5 mL) and glacial acetic acid (25 mL). The solution was diluted with benzene (25 mL) under ice cooling. Into the solution of above reagent compound Ib (2.68 g, 0.01 mol) or XIb (2.96 g, 0.01 mol) in benzene (5 mL) was added dropwise with stirring. The reaction mixture was kept below 15C. The compound Ib or XIb was consumed within 2 hr. as observed by TLC. The reaction mixture was diluted with water, neutralized with aqueous sodium hydroxide solution, extracted with ether and dried. After evaporation of solvent and crys-

tallization from hexane, a white solid product was obtained, VI (2.25 g, ~ 84%, m.p. 64-65C, Lit. m.p. 65-66C)[16]; LXXIII (2.47 g, ~ 83.5, m.p. 68C).

Analysis: (VI) Calcd. for $C_{17}H_{30}O_3$: C, 72.29; H, 10.71; Found: C, 72.23; H, 10.65%. UV(λmax.): 220 nm. IR(KBr): 1665 (-CO-CH=CH-), 1645 (-CH=CH-) and 995 (trans-unsaturation). NMR($CDCl_3$): 2.95 d (1H, $-CH=CH-CO_2CH_3$, J=16 Hz), 3.46 d (1H, $-CH=CH-CO_2CH_3$, J=16 Hz), 7.5 m ($-CH_2-\overset{O}{\overset{\|}{C}}-$), 8.7 (br,s, 20H) and 9.1 (t, 3H). Mass: m/z 282 (M^+).

(LXXIII) Calcd. for $C_{19}H_{34}O_3$: C, 73.5; H, 10.03; Found: C, 73.35; H, 9.98%. IR and NMR data are similar as that of VI.

Peracid Oxidation of Ib and XIb

Reaction of methyl trans-2-enoate of C_{16} and C_{18} chain length Ib and XIb (Ib, 3 g, 0.001 mol) was carried out in chloroform (100 mL)[31] using m-CPBA (6 g, 0.035 mol) as oxidant. The reaction was kept at room temperature for 10 days. After removal of solvent the reaction mixture was extracted with ether and washed with 10% solution of sodium sulphite (3x20 mL) to destroy excess of peroxide. The organic layer was then shaken with 5% aqueous sodium bicarbonate to remove m-chlorobenzoic acid and dried over Na_2SO_4. The reaction mixture of Ib (2.8 g) was chromatographed over a column of silica gel (45 g). The elution was carried out with pet. ether containing increasing amount of ethyl ether. 25 mL fraction was collected and tested. Elution with pet. ether gave the starting material Ib (0.84 g, 30%).

Subsequent elution with pet. ether-ether (97:3, v/v) gave LVIb (1.82 g, ~ 65%) and LVII (0.11 g, ~ 3.8%).

Methyl trans-2,3-epoxyhexadecanoate (LVIb, m.p. 35-36C)

Analysis: Calcd. for $C_{17}H_{32}O_3$: C, 71.78; H, 11.34; Found: C, 71.69; H, 11.30%. IR(nujol): 1750, 1735 (ester carbonyl), 1285, 1240 (C-C and C-O, ring breathing vibration), 1190, 1175 (C-O) and 890 (trans-epoxy group). IR($CHCl_3$): 1745, 1725 ($\underline{COO}CH_3$, second band as a shoulder) and 885. NMR(CCl_4): 6.24 (s, 3H), 6.88 broad signal (2H, -CH(O)-CH-CO_2CH_3), 8.7 (br,s, 24H) and 9.12 (t, 3H).
^{13}C NMR($CDCl_3$): 52.92 (OCH_3), 169.72 (C_1), 52.22 (C_2), 58.45 (C_3), 31.47 (C_4), 25.73 (C_5), 29.68 (C_6), 29.52 (C_7-C_{12}), 29.36 (C_{13}), 31.96 (C_{14}), 22.69 (C_{15}) and 14.08 (C_{16}). Mass: m/z 284 (M^+).

Methyl cis-3,4-epoxyhexadecanoate (LVIII, m.p. 44-45C)

Analysis: Calcd. for $C_{17}H_{32}O_3$: C, 71.78; H, 11.34; Found: C, 71.74; H, 11.32%. IR(CCl_4): 1730 (-$\underline{COO}CH_3$), 1265, 1225, 1190, 1165, 1030 (C-O) and 840 (cis-epoxy group). NMR($CDCl_3$): 6.27 (s, 3H), 6.73 m (1H, -CH(O)-CH-$CH_2CO_2CH_3$), 7.04 m (1H, -CH(O)-CH-$CH_2CO_2CH_3$), 7.46 d (2H, -CH_2-CO_2CH_3, J=2 Hz), 8.74 (br,s, 22H) and 9.12 (t, 3H).

^{13}C NMR($CDCl_3$): 52.49 (OCH_3), 171.02 (C_1), 33.74 (C_2), 51.84 (C_3), 56.66 (C_4), 29.63 (C_5), 26.43 (C_6), 29.47 (C_7-C_{12}), 29.36 (C_{13}), 31.96 (C_{14}), 22.69 (C_{15}) and 14.08 (C_{16}). Mass: m/z 284 (M^+).

Similarly, reaction mixture of XIb was separated into LVIIb and LIX with the help of silica gel column.

Methyl trans-2,3-epoxyoctadecanoate (LVIIb) (m.p. 42-43C)

Analysis: Calcd. for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61; Found: C, 73.00; H, 11.59%. IR and NMR data are similar as that of LVIb.

Methyl cis-3,4-epoxyoctadecanoate (LIX) (m.p. 49.5)

Analysis: Calcd. for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61; Found: C, 72.98; H, 11.60%. IR and NMR spectral data are same as these of LVIII.

When similar epoxidation was performed by refluxing the reactants Ib and XIb with m-CPBA in methylene dichloride for 58 hr., an improved yield of epoxides (LVIb, LVIIb, ~70%) and (LVIII, LIX, ~4.3%) was obtained.

Acetolysis of LVIb and LVIIb

Compounds LVIb and LVIIb (0.5 g, each) were boiled separately with glacial acetic acid (10 mL) for 10 hr. The

mixture was then saponified, acidified and diluted with water and extracted repeatedly with ether. The combined ethereal extracts were dried. After the removal of solvent in rotatony evaporator, the crude dihydroxy acids on crystallization from acetone and pet. ether (3:1, v/v) gave solid products LXa (m.p. 102C, Lit. m.p. 103-104C)[14] and LXIa (m.p. 105C, Lit. m.p. 106)[7]. Methyl ester of these dihydroxy acids were prepared by the usual method.

Analysis: (LXb) Calcd. for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33; Found: C, 67.48; H, 11.31%.

(LXIb) Calcd. for $C_{19}H_{38}O_4$: C, 69.05; H, 11.59; Found: C, 49.04; H, 11.56%. IR(nujol): 3400 (OH), 1730 (-$\underline{C}OOCH_3$) and 1120, 1070 (C-O).

The epoxy esters LVIb and LVIIb (50 mg, each) were refluxed with methanolic potassium hydroxide (1M, 40 mL) for 30 minutes. The solution was neutralized with dilute hydrochloric acid, diluted with water and extracted immediately with ether. The solid acids (LVIa, m.p. 81C) (LVIIa, m.p.86C, Lit. m.p. 88-86C)[31] were crystallized from pet. ether. IR(nujol): 3380-3160 (CO$\underline{O}$H), 1730, 1705 ($\underline{C}$OOH), 1070 and 890.

Peracid Oxidation of IVb

To the solution of methyl 4-hydroxy-trans-2-hexadecenoate (IVb, 0.68 g, 0.0021 mol) in chloroform (10 mL), m-CPBA (0.365 g, 0.0021 mol) in chloroform (5 mL) was added with

shaking. TLC examination of the reaction product after 48 hr. showed only minor change in starting material. Therefore, it was refluxed for 4 hr. on a water bath. The mixture was cooled and the solvent was removed under reduced pressure. The reaction mixture was worked-up adopting the earlier procedure and was chromatographed over a column of silica gel (10.0 g) using pet. ether containing increasing amounts of ethyl ether as eluent. Elution with pet. ether (88:12, v/v) gave the starting material (IVb, 0.36 g, 66.6%, m.p. 56-57C).

Further elution with a mixture of pet. ether-ether (86:14, v/v) gave methyl 4-hydroxy-trans-2,3-epoxyhexadecanoate (LXII, 0.172 g, ~32%, m.p. 48-49C).

Analysis: Calcd. for $C_{17}H_{32}O_4$: C, 67.96; H, 10.73; Found: C, 67.89; H, 10.72%. IR(CCl_4): 3380-3290 (OH), 1730 ($\underline{COO}CH_3$), 1275, 1230 (C-C and C-O bonds ring breathing vibration) and 880 sharp, 830 (trans-epoxy ring). NMR($CDCl_3$): 6.25 (s, 3H), 6.28 br,m (1H, $-\underline{CH_2}-OH$, merged in parts with signal of proton of methyl ester), 6.6 broad signal (1H, $-\underline{CH}-\underset{O}{\diagdown\diagup}-\underline{CH}-CO_2CH_3$), 6.8 broad signal (1H, $-\underline{CH}-\underset{O}{\diagdown\diagup}-CH-CO_2CH_3$), 7.58 br,s (1H, $O\underline{H}$, D_2O exchangeable), 8.7 (br,s, 22H) and 9.14 (t, 3H). Mass: m/z 282 (M-18).

The same reaction, when carried out with 2 mol of m-CPBA afforded VI (0.084 g, ~15.3%, m.p. 64-68C, Lit. m.p. 65-66C)[16] along with LVIb (0.36 g, ~ 65.8%). Elemental and spectral data of VI resembled with those of compound VI in scheme 11.

Peracid Oxidation of LIV

trans-2-Octadecen-1-ol (LIV, 0.5 g, 0.0018 mol) was treated with m-CPBA (0.32 g, 0.00185 mol) in chloroform (15 mL) at room temperature for 30 minutes. The reaction mixture after usual workup and crystallization from pet. ether afforded a solid product (LXIII, 0.51 g, ~100%, m.p. 73-74C).
Analysis: Calcd. for $C_{18}H_{36}O_2$: C, 75.99; H, 12.75; Found: C, 75.82; H, 12.70%. IR(CCl_4): 3270 (OH), 1450, 1050 (C-O) and 860 (trans-epoxy ring). NMR(CCl_4): 6.46 m (2H, $-C\underline{H}_2-OH$), 6.52 br,s (1H, $-CH_2-O\underline{H}$, D_2O exchangeable), 7.5 m (2H, $-C\underline{H}-C\underline{H}_2-$, O), 8.68 (br,s, 28H) and 9.12 (t, 3H). Mass: m/z 284 ($M^{\ddagger}$).

Peracid Oxidation of VI and LV

A solution of methyl 4-oxo-trans-2-hexadecenoate (VI, 0.3 g, 0.001 mol) in chloroform (10 mL) was allowed to react with m-CPBA (0.9 g, 0.005 mol in 10 mL $CHCl_3$) at room temperature for 16 days. Similarly methyl 4-oxo-trans-2-octadecenoate (LV) was treated with m-CPBA. Reaction products were worked up as described earlier. Liquid product (0.29 g) was passed through a column of silica gel (10 g). Elution with pet. ether-ether (99:1, v/v) yielded product LXIV (0.27 g, 90%).
Analysis: (LXIV) Calcd. for $C_{17}H_{30}O_4$: C, 68.42; H, 10.13; Found: C, 68.08; H, 10.10%.

(LXV) Calcd. for $C_{19}H_{34}O_4$: C, 69.9; H, 10.48; Found: C, 69.7; H, 10.32%. IR(CCl_4): 1735, 1725, 1710 ($-CH_2-\overset{O}{\overset{\|}{C}}-CH=CH-\overset{O}{\overset{\|}{C}}-O-CH_3$), 1645 (-CH=CH-), 975 (trans-unsaturation), 1220, 1165, 1150 (C-O-C) and 1105, 1030, 1005 (C-O). NMR($CDCl_3$): 3.21 s (2H, $-COC\underline{H}=C\underline{H}-CO_2CH_3$), 5.87 t ($-C\underline{H}_2O-$, J=16 Hz), 8.7 (br,s, $-(C\underline{H}_2)_n-$ and 9.12 (t, 3H). Mass: (LXIV) m/z 298 (M^+).

Preparation and Isolation of Epoxy Esters (LXVI-LXVIII, LVIb, LXIII)

Methyl undecenoate and methyl oleate (0.005 mol, each) were treated with m-CPBA (0.005 mol) in chloroform (10 mL) at 5C and kept at room temperature for 4 hr. The reaction mixtures were workedup in the usual way. Evaporation of solvent furnished LXVI and LXVII in quantitative yields (~97%). Compounds LVIb and LXIII were prepared as described earlier.

Compound LXVIII was isolated from Vernonia anthelmintica seed oil. The oil was transesterified with 0.5% sodium methoxide solution. After cooling the solution it was acidified with acetic acid and the usual workup yielded LXVIII which was later purified by column chromatography.

Methyl 10,11-epoxyundecanoate (LXVI)

Analysis: Calcd. for $C_{12}H_{22}O_3$: C, 67.26; H, 10.35; Found: C, 67.21; H, 10.32%. IR(CCl_4): 1740 ($\underline{CO}OCH_3$) and 835 (cis-

epoxy group). NMR(CCl_4): 7.4-7.6 m (3H, CH_2 - CH-), 6.32 (s, 3H) and 8.72 (br,s, 14H).

Methyl cis-9,10-epoxystearate (LXVII)

Analysis: Calcd. for $C_{19}H_{36}O_3$: C, 73.03; H, 11.61; Found: C, 73.00; H, 11.58%. IR(CCl_4): 840 and 820 (cis-epoxy group). NMR(CCl_4): 7.31 m (2H, -CH - CH-), 7.78 (m, 2H), 8.68 (br,s, 26H) and 9.12 (t, 3H).

Methyl cis-12,13-epoxy-cis-9-octadecenoate (LXVIII)

Analysis: Calcd. for $C_{19}H_{34}O_4$: C, 73.50; H, 10.04; Found: C, 73.09; H, 9.89%. IR(CCl_4): 1740 ($-COOCH_3$), 840 and 820 (cis-epoxy group). NMR(CCl_4): 4.58 m (2H, -CH=CH-), 7.31 m (2H, -CH - CH-), 7.78 m (4H, $-CH_2-CH=CH-(CH_2)_6-CH_2-CO_2CH_3$) and 7.94 m (2H, $-CH=CH-CH_2-$, a part merged with signal at 7.78).

Reaction of Long-Chain Epoxy Compounds (LXVI-LXVIII, LVIb, LXIII) with CTMS

As a general method the epoxy compounds and CTMS (1:1 molar ratio) were allowed to react in presence of diethyl ether (25 mL) at room temperature for 2-3 minutes. Exception to the duration of reaction was adopted in case of epoxy compound LVIb i.e. for 15 minutes. The reaction mixture was washed with water and dried over Na_2SO_4. Silica gel column chromatographic separation yielded the corresponding chlorohydrins detailed as under.

Methyl 11-chloro-10-hydroxyundecanoate (LXIXa) (m.p. 47-48C)

Analysis: Calcd. for $C_{12}H_{23}O_3Cl$: C, 57.47; H, 2.24; Found: C, 57.02; H, 9.12%. IR(nujol): 3360 (OH), 1730 ($\underline{CO}OCH_3$) and 730 (C-Cl). NMR(CCl_4): 6.2-6.5 br,m (3H, $-C\underline{H}_2-Cl$ and $-C\underline{H}-OH$, a part merged with ester protons signal at 6.3), 6.78 s (1H, $-O\underline{H}$, disappeared on addition of D_2O), 7.7 m (2H, $-C\underline{H}_2-CO_2CH_3$) and 8.65 (br,s, 14H). Mass: m/z 251/253 (M+1).

Methyl 9(10)-chloro-10(9)-hydroxyoctadecanoate (LXX)

Analysis: Calcd. for $C_{19}H_{37}O_3Cl$: C, 65.39; H, 10.69; Found: C, 65.30; H, 10.65%. IR(CCl_4): 3440 (OH), 1740 ($\underline{CO}OCH_3$), 720 (C-Cl). NMR(CCl_4): 6.05 m (1H, $-C\underline{H}-Cl$), 6.4 m (1H, $-C\underline{H}-OH$), 7.45 s (1H, $O\underline{H}$, D_2O exchangeable), 7.7 (m, 2H), 8.7 (br,s, 26H) and 9.1 (t, 3H).

Methyl 2-chloro-3-hydroxyhexadecanoate (LXXIa)

Analysis: Calcd. for $C_{17}H_{33}O_3Cl$: C, 63.63; H, 10.36; Found: C, 63.60; H, 10.24%. IR(CCl_4): 3470 (OH), 1735 ($\underline{CO}OCH_3$), 825 (C-Cl). NMR(CCl_4): 5.20 m (1H, $-C\underline{H}-Cl$), 5.9 m (1H, $-C\underline{H}-OH$), 6.21 (s, 3H), 7.12 br,s (1H, $-O\underline{H}$, disappeared on addition of D_2O), 8.7 (br,s, 24H) and 9.1 (t, 3H). Mass: m/z 321/323 (M+1).

Methyl 3-chloro-2-hydroxyhexadecanoate (LXXIb) (m.p. 40C, Lit. m.p. 41-42C)[13]

Analysis: Calcd. for $C_{17}H_{33}O_3Cl$: C, 63.63; H, 10.36; Found: C, 63.59; H, 10.31%. IR(CCl_4): 3930 (OH), 1740 ($\underline{CO}OCH_3$), 810

(C-Cl). NMR(CCl_4): 3.8 m (1H, -C<u>H</u>-Cl), 6.0 m (1H, -C<u>H</u>-OH), 6.23 (s, 3H), 6.9 br,s (1H, -O<u>H</u>, D_2O exchangeable), 8.7 (br,s, 24H) and 9.12 (t, 3H). Mass: m/z 321/323 (M+1).

<u>2(3)-chloro-3(2)-hydroxyoctadecan-1-ol (LXXII) (m.p. 78-79C)</u>

Analysis: Calcd. for $C_{18}H_{37}O_2Cl$: C, 67.36; H, 11.62; Found: C, 67.07; H, 11.53%. IR(nujol): 3460 (OH), 810 (C-Cl). NMR ($CDCl_3$): 5.68-6.48 m (4H, -C<u>H</u>-C<u>H</u>-CH_2-OH), 7.63 s (2H, 2xO<u>H</u>), exchangeable with D_2O), 8.63 (br,s, 28H) and 9.1 (t, 3H).

<u>12(13)-chloro-13(12)-hydroxy-cis-9-octadecenoate (LXXIII)</u>

Analysis: Calcd. for $C_{19}H_{35}O_3Cl$: C, 65.78; H, 10.17; Found: C, 65.72; H, 10.12%. IR(neat): 3460 (OH) and 715 (C-Cl). NMR (CCl_4): 4.58 m (2H, -C<u>H</u>=C<u>H</u>-), 6.05 m (1H, -C<u>H</u>-Cl), 6.5 m (1H, -C<u>H</u>-OH, in parts merged with the signal of methyl ester at 6.37), 7.62 s (1H, -O<u>H</u>, D_2O exchangeable), 7.8 (m, 6H), 8.65 (br,s, 18H) and 9.12 (t, 3H).

<u>Reaction of trans-2-Octadecen-1-ol (LIV) with Iodine Azide</u>

Compound LIV was treated with iodine azide according to procedure of Fowler <u>et al.</u>[47] with some modification. To a cooled stirred slurry of sodium azide (0.5 g) in acetonitrile (80 mL), iodine monochloride (0.2 mL) was added slowly over a period of 10 minutes. The reaction mixture was stirred for further 10 minutes and then compound LIV (1.0 g, 0.003 mol) in

dry ether (10 mL) was added slowly. Reaction mixture was stirred for additional 24 hr. at room temperature (The compound LIV was insoluble in acetonitrile thats why ethereal solution of LIV was used). A red brown slurry was obtained and was extracted with ether in three portions. The combined ethereal layer was washed with 2% solution of sodium thiosulphate, leaving a colourless ethereal solution which was again washed with water and dried over Na_2SO_4. Evaporation of solvent gave a dark brown liquid (1.64 g), which showed two spots on TLC. The reaction product (1.6 g) was purified by column chromatography using silica gel (35 g) and mixture of pet. ether-ether as eluting solvent. Elution with pet. ether-ether (92:8, v/v) gave the starting material LIV (0.049 g, m.p. 46C). Further elution with pet. ether-ether (89:11, v/v) gave a viscous brown liquid (LXXIVa,b, 1.51 g, ~ 97%, positive Beilstein test). Analysis: Calcd. for $C_{18}H_{36}OIN_3$: C, 49.42; H, 8.92; N, 9.60; Found: C, 49.35; H, 8.70; N, 9.35%. IR(nujol): 3380-3260 (OH) and 2100 (N_3). NMR($CDCl_3$): 5.92 m (1H, $-\underset{I}{CH}-I$), 6.46 m (2H, $-CH_2OH$), 6.94 br,s (1H, OH, disappeared on D_2O shake), 6.68 m (1H, $-\underset{N_3}{CH}-N_3$), 8.65 (br,s, 28H) and 9.12 (t, 3H). Mass:m/z 437 (M^+).

Dehydroiodination of LXXIVa,b

Compound LXXIVa,b (0.9 g, 0.002 mol) was treated with KOH (1.8 g) in dry methanol (5 mL) with continuous stirring at room temperature for 14 hr. Reaction mixture was workedup as

described above. Evaporation of solvent gave a yellow liquid (0.61 g, negative Beilstein test). Silica gel (8.0 g) column chromatographic purification furnished a light yellow liquid (LXXVa,b, 0.58 g, ~ 100%).

Analysis: Calcd. for $C_{18}H_{35}ON_3$: C, 69.85; H, 11.39; N, 13.57; Found: C, 69.50; H, 11.31; N, 12.97%. IR(nujol): 3330 (OH), 2100 (N_3) and 1650 (-HC=CH-). NMR(CCl_4): 4.95 t (1H, $-C\overset{H}{\equiv}C-$, J=7 Hz), 6.48 s and 6.52 d (2H, $-C\equiv C-CH_2OH$, J=6 Hz), 6.65 br, s (1H, O<u>H</u>, disappeared on D_2O shake), 7.95 m (2H, $-CH_2-C\equiv C-CH_2OH$), 8.7 (br,s, 26H), 9.12 (t, 3H). Mass: m/z 309 (M^+).

<u>Preparation of Methyl 2,3-dibromohexadecanoate[5] (IIIb, m.p. 36-37C)</u>

Methyl <u>trans</u>-2-hexadecenoate (Ib, 5.0 g, 0.018 mol) was treated with a cold solution of bromine (8.0 g, 0.05 mol) in dry alcohol-free chloroform (45 mL) for 2 hr. Stirring was continued at room temperature for 6 hr. The reaction mixture was then warmed at 50C for 12 hr. After evaporation of solvent, reaction product was dissolved in ether and excess bromine was first destroyed with aqueous sodium thiosulphate and then washed and dried. Evaporation of the ether gave a solid product (~ 7.6 g) which on crystallization from pet. ether yielded IIIb (positive Beilstein test, 6.9 g, ~ 91%).

Analysis: Calcd. for $C_{17}H_{32}O_2Br_2$: C, 47.68; H, 7.53; Found:

C, 47.59; H, 7.50%. IR(CCl_4): 1740 ($\underline{CO}OCH_3$) and 650 (C-Br). NMR(CCl_4): 5.76 m (2H, -$\underset{Br}{\underline{CH}}$ - $\underset{Br}{\underline{CH}}$-), 6.28 (s, 3H), 8.78 (br,s, 24H) and 9.13 (t, 3H).

Reaction of IIIb with Ammonia at 0C

Compound IIIb (4.0 g, 0.007 mol) in methanol (5 mL) was added to methanol (60 mL) saturated with dry ammonia at 0C. The mixture was stirred for 12 hr. at this temperature. Solvent was removed and the products were taken in ether, washed and dried. The crude product (3.4 g) was passed over silica gel (45 g) column. Elution with pet. ether gave the starting material (IIIb, 0.11 g, ~3.4%). Further elution with pet. ether-ether (95.5:0.5, v/v) gave methyl 2-bromo-2-hexadecenoate (LXXVI, 3.12 g, ~93%).
Analysis: Calcd. for $C_{17}H_{31}O_2Br$: C, 58.78; H, 8.99; Found: C, 58.65; H, 8.87%. IR(CCl_4): 1725, 1715 ($\underline{CO}OCH_3$), 1610 (-HC=CH-) and 645 (C-Br). NMR(CCl_4): 3.39 t (1H, -$\underline{CH}$=C-CO_2CH_3, J=8 Hz) and 7.5 m (2H, -$\underline{CH_2}$CH=C-), 8.7 (br,s, 22H) and 9.12 (t, 3H).

Reaction of LXXVI with Ammonia at 25C

Compound LXXVI (2.5 g, 0.007 mol) in methanol (5 mL) was added to methanol (35 mL) saturated with ammonia at 0C. The reaction mixture was stirred at 5C for 3 hr. and then at 25C for 10 hr. After evaporation of solvent, it was workedup as

usual. Evaperation of solvent yielded a solid (2.0 g) showing four spots on TLC. Silica gel (45 g) chromatographic separation yielded four homegeneous products: (LXXVII, ~ 5%; LXXVIII, ~64%; LXXIX, ~ 24% and LXXX, ~ 3%) using a mixture of pet. ether-ether (95:5, 92:8, 90:10, v/v) and benzene-chloroform (60:40, v/v) respectively.

Methyl-2-amino-2-hexadecanoate (LXXVII)

Analysis: Calcd. for $C_{17}H_{33}NO_2$: C, 72.08; H, 11.74; N, 4.94; Found: C, 71.88; H, 11.70; N, 4.45%. IR(CCl_4): 3400-3300 (NH_2), 1730, 1720 (-COOCH$_3$), 1620 (-HC=CH-), 1240, 1040 (C-N), 1000 (C-O) and 750. NMR(CCl_4): 2.77 t (1H, -HC=C-CO_2CH_3, J=7 Hz), 6.13 s (2H, =C-NH_2), 6.21 (s, 3H), 7.75 m (2H, -CH_2CH=C-), 8.7 (br,s, 22H) and 9.12 (t, 3H).

Methyl trans-2,3-epiminohexadecanoate (LXXVIII, m.p. 39-40C)

Analysis: Calcd. for $C_{17}H_{33}NO_2$: C, 72.08; H, 11.74; N, 4.94; Found: C, 71.90; H, 11.62; N, 4.4%. IR(KBr): 3280, 895 (trans-aziridine ring) and 1730 (ester carbonyl). IR($CHCl_3$): 3270, 880 (aziridine ring). NMR(CCl_4): 7.84 br, signal (2H, -CH - CH-) and 8.02 s (1H, >NH), 6.28 (s, 3H), 8.7 (br,s, 24H) and 9.12 (t, 3H). Mass: m/z 283 (M^+).

Methyl cis-2,3-epiminohexadecanoate (LXXIX, m.p. 56-57C)

Analysis: Calcd. for $C_{17}H_{33}NO_2$: C, 72.08; H, 11.74; N, 4.94; Found: C, 71.87; H, 11.65; N, 4.81%. IR(KBr): 3170, 845 (cis-

aziridine ring) and 1735 ($\underline{C}OOCH_3$). NMR($CDCl_3$): 6.27 (s, 3H), 7.56 d (1H, -CH - $\underline{C}H$-CO_2CH_3, J=6 Hz), 7.91 m (1H, -$\underline{C}H$ - CH-CO_2CH_3), 8.21 s (1H, $>N\underline{H}$), 8.71 (br.s, 24H) and 9.12 (t, 3H). Mass: m/z 283 (M^+).

trans-2,3-Epiminohexadecamide (LXXX, m.p. 116-117C)

Analysis: Calcd. for $C_{16}H_{32}N_2O$: C, 71.59; H, 12.01; N, 10.43; Found: C, 71.45; H, 11.90; N, 10.08%. IR(KBr): 3380, 3180 ($-NH_2$), 3275, 855 (trans-aziridine ring), 1650 ($-\underline{C}ONH_2$) and 1620 (NH_2 deformation). IR($CHCl_3$): 3500, 3380 ($-NH_2$), 3280, 850 (trans-aziridine ring), 1675 ($-\underline{C}ONH_2$) and 1585 (NH_2 deformation, weak). Mass: m/z 268 (M^+).

Reaction of LXXVIII with Acetic acid[93]

A solution of LXXVIII (0.2 g, 0.7 mmol) in glacial acetic acid (4 mL) was heated at reflux for 1 hr. Acetic acid was evaporated *in vacuo*. The residue was dissolved in chloroform, washed, dried and evaporated to dryness. The final product (0.242 g) showed two spots on direct TLC plate. It was chromatographed over silica gel (5.0 g) column using pet. ether-ether as moving phase. Elution with pet. ether-ether (60:40, v/v) yielded methyl 2-acetamide-3-acetoxyhexadecaneate (LXXXII) as a semisolid (0.03 g, ~12%).

Analysis: Calcd. for $C_{21}H_{39}NO_5$: C, 65.42; H, 10.20; N, 3.63; Found: C, 65.40; H, 10.19; N, 3.59%. IR($CHCl_3$): 3410 ($>NH$),

1740 (-O<u>C</u>OCH$_3$), 1730 (-<u>C</u>OOCH$_3$), 1685 (-NH<u>C</u>OCH$_3$) and 1268 (acetate). NMR(CDCl$_3$): 5.8 m (1H, -C<u>H</u>-NHCOCH$_3$), 6.15 m (1H, -C<u>H</u>-OCOCH$_3$), 6.22 (s, 3H), 7.95 (s, 3H), 7.98 (s, 3H), 8.1 m (1H, >N<u>H</u>), 8.7 (br,s, 24H) and 9.12 (t, 3H). Mass: m/z 385 (M^+).

Subsequent elution with pet. ether-ether (50:50, v/v) gave methyl 2-acetamido-3-hydroxyhexadecanoate (LXXXIII, 0.21 g, ~ 88%, m.p. 84C).

Analysis: Calcd. for $C_{19}H_{37}NO_4$: C, 66.43; H, 10.86; N, 4.08; Found: C, 66.42; H, 10.83; N, 4.07%. IR($CHCl_3$): 3520 (OH), 3440, 3335 (>NH), 1735 (-<u>C</u>OOCH$_3$), 1680 (-NH<u>C</u>OCH$_3$), 1620 (>NH, def., weak), 1260 (acetate) and 1070 (C-O). NMR(CDCl$_3$): 4.3 m (1H, O<u>H</u>), 5.68 m (1H, -C<u>H</u>-NHCOCH$_3$), 6.19 (s, 3H), 6.2 m (1H, -C<u>H</u>-OH), 7.97 s (3H, -NHCOC<u>H</u>$_3$), 8.08 m (1H, >N<u>H</u>), 8.71 (br,s, 24H) and 9.1 (t, 3H). Mass: m/z 343 (M^+).

References

1. ApSimon, J., The Total Synthesis of Natural Products, Vol.2, John Wiley and Sons, New York (1973).

2. Maercker, A., Org. React. 14:270 (1965).

3. Tulloch, A.P., Lipids 6:641 (1971).

4. LeSueur, H.R., J. Chem. Soc. 1708 (1904).

5. Myers, G.S., J. Am. Chem. Soc. 73:2100 (1951).

6. Murty, G.V.L.N. and T.R. Seshadri, Chem. Abstr. 34:7744 (1940).

7. Palameta, B. and M. Prostenik, Tetrahedron 19:1463 (1963).

8. Barton, D.H.R. and E. Miller, J. Am. Chem. Soc. 72:1066 (1950).

9. Dhar, M.L., E.D. Hughes, C.K. Ingold, A.M.M. Mandour, G.A. Maw and L.I. Woolf, J. Chem. Soc. 2093 (1948).

10. Ahmad Jr., M.S., M.U. Ahmad and S.M. Osman, JAOCS 56: 867 (1979).

11. Barve, J.A. and F.D. Gunstone, Chem. Phys. Lipids 7:311 (1971).

12. Gunstone, F.D. and I.A. Ismail, ibid. 1:264 (1967).

13. Ansari, A.A., F. Ahmad and S.M. Osman, JAOCS 53:541 (1976).

14. Ansari, A.A. and S.M. Osman, ibid. 53:118 (1976).

15. Husain, S.R., F. Ahmad and M. Ahmad, ibid. (In Press).

16. Ahmad, M.U., M.S. Ahmad, Jr. and S.M. Osman, ibid. 55: 491 (1978).

17. Nakayama, M., S. Shinke, Y. Matsushita, S. Ohira and S. Hayashi, Bull. Chem. Soc. (Japan) 52:184 (1979).

18. Frighetto, N., C.L.P. Silveira, F.A.M. Reis, E.G. Magalhaes and E.A. Ruveda, Chem. Phys. Lipids 22:115 (1978).

19. MacMillan, J.H. and S.S. Washburne, Org. Magn. Resonance 6:250 (1974).

20. Simmons, H.E. and R.D. Smith, J. Am. Chem. Soc. 80:5323 (1958).

21. Setser, D.W. and B.S. Rabinovitch, J. Org. Chem. 26:2985 (1961).

22. Wood, R. and R. Reiser, JAOCS 42:315 (1965).

23. Gunstone, F.D. and B.S. Perera, Chem. Phys. Lipids 10: 303 (1973).

24. Ahmad, Jr., M.S. and S.M. Osman, JAOCS 57:363 (1980).

25. Dauben, W.G. and G.H. Berezin, J. Am. Chem. Soc. 85:468 (1963).

26. Chan, J.H.H. and B. Rickborn, ibid. 90:6406 (1968).

27. Simmons, H.E., T.L. Cairns, S.A. Veaduchick and C.M. Hoiness, Org. React. 20:1 (1973).

28. Ratier, M., M. Castaing, J. Godel and M. Pereyre, J. Chem. Research(S) 5:179 (1978).

29. Swern, D., Organic Peroxides (ed. D. Swern), Vol.2, John Wiley and Sons, Inc. (1971).

30. Artamonov, P.A., Zhur. Obschei. Khim. 28:1355 (1958).

31. Gunstone, F.D. and F.R. Jacobsberg, Chem. Phys. Lipids 9:26 (1972).

32. Ansari, A.A., F. Ahmad and S.M. Osman, Fette Seifen Anstrichm. 79:328 (1977).

33. Ansari, A.A., B. Schmitz and H. Egge, Chem. Phys. Lipids 29:45 (1981).

34. Martin, L.S. and J. English, J. Am. Chem. Soc. 82:4891 (1960).

35. Cella, J.A., J.P. McGrath, J.A. Kelley, O.El Soukkay and L. Hilpert, J. Org. Chem. 42:2077 (1977).

36. Johnson, M.R., T. Nakata and Y. Kishi, Tetrahedron Lett. 4343 (1979).

37. Johnson, M.R. and Y. Kishi, ibid. 4347 (1979).

38. Hasan, I. and Y. Kishi, ibid. 4229 (1980).

39. van Haard, P.M.M., L. Thijs and B. Zwanenburg, ibid. 803 (1975).

40. Brouwer, A.C., L. Thijs and B. Zwanenburg, ibid. 807 (1975).

41. Thijs, L., F.L.M. Smeets, P.J.M. Cilliaan, J. Harmsen and B. Zwanenburg, Tetrahedron 36:2141 (1980).

42. Thijs, L. and B. Zwanenburg, ibid. 36:2145 (1980).

43. Gilbert, J., M.J. Shepherd, J.R. Startin and J. Eagles, Chem. Phys. Lipids 28:61 (1981).

44. Fowler, F.W., A. Hassner and L.A. Levy, J. Am. Chem. Soc. 89:2077 (1967).

45. Hassner, A., Acc. Chem. Res. 4:9 (1971).

46. Cambie, R.C., J.L. Jurlina, P.S. Rutledge, B.E. Swedlund and P.D. Woodgate, J. Chem. Soc., Perkin Trans.I 327 (1982).

47. Foglia, T.A., P.A. Barr and G. Maerker, JAOCS 49:414 (1972).

48. Ali, M.L., Ph.D. Thesis, A.M.U., Aligarh (1980).

49. Shin, C., Y. Yonezawa, K. Uneki and J. Yoshimura, Bull. Chem. Soc.(Japan) 52:1687 (1979).

50. Yonezawa, Y., C. Shin, Y. One and J. Yoshimura, ibid. 53:2906 (1980).

51. Shin, C., Y. Yonezawa, K. Watanabe and J. Yoshimura, ibid. 54:3811 (1981).

52. Shin, C., Y. Yonezawa, H. Nazakawa, K. Nanjo and J. Yoshimura, ibid. 45:3598 (1972).

53. Hasan, S.Q., I. Ahmad, F. Ahmad and S.M. Osman, JAOCS (In Press).

54. Nasirullah, F. Ahmad and S.M. Osman, Fette Seifen Anstrichm. (In Press).

55. Moureu, M., P. Chevin and L. Petil, Bull. Soc. Chim. (France) 1573 (1936).

56. Gundermann, K.D., G. Holtmann, H.J. Rose and H. Schulze, Chem. Ber. 93:1632 (1960).

57. Berlin, K.D., L.G. Williams and O.C. Dermer, Tetrahedron Lett. 873 (1968).

58. Kyburz, E., H. Ela, St. Majnoni, G. Englert, C. von Planta, A. Furst and Pl.A. Plattner, Helv. Chim. Acta 49:359 (1966).

59. Lown, J.W., T. Itoh and N. Ono, Cand. J. Chem. 51:856 (1973).

60. Harada, K. and I. Nakamura, J. Chem. Soc., Chem. Commun. 522 (1978).

61. Abbot, G.G. and F.D. Gunstone, Chem. Phys. Lipids 7:290 (1971).

62. Gunstone, F.D. and M. Lie Ken Jie, ibid. 4:139 (1970).

63. von Jenny, E.F. and C.A. Grob, Helv. Chim. Acta 36:1456 (1953).

64. Novak, L., G. Bann, Y. Maroafalvi and C. Szantak, Tetrahedron Lett. 487 (1978).

65. House, H.O. and J.W. Blaker, J. Am. Chem. Soc. 80:6389 (1958).

66. Colthup, N.B., L.H. Daly and S.E. Wiberley, Introduction to Infrared and Raman Spectroscopy, Academic Press Inc. (NY), p.315 (1975) and references cited therein.

67. Odham, G. and E. Stenhagen, Biochemical Applications of Mass Spectrometry (ed. G.R. Waller), John Wiley and Sons, New York, p.211 (1972).

68. Rhoads, S.J., J.K. Chattopadhyay and E.E. Waali, J. Org. Chem. 35:3352 (1970).

69. Fioriti, J.A. and R.J. Sims, J. Chromatog. 32:761 (1968).

70. Tokumaru, K., O. Shimamura and M. Fukuyama, Bull. Chem. Soc.(Japan) 35:1673 (1962).

71. Gruselle, M., M. Tichy and D. Lefort, Tetrahedron 28: 3885 (1972).

72. Celia, J.A. and J.P. McGrath, Tetrahedron Lett. 4115 (1975).

73. Hawthorne, M.F., Anal. Chem. 28:540 (1956).

74. Heywood, D.L. and Phillips, B., J. Org. Chem. 25:1699 (1960).

75a. Lavigne, A.A., J. Tancrede, R.M. Pike and C.T. Tabit, J. Organomet. Chem. 15:57 (1968).

b. Murata, S., M. Suzuki and R. Noyori, J. Am. Chem. Soc. 101:2738 (1979).

c. Kraus, G.A. and K. Frazier, J. Org. Chem. 45:2579 (1980).

d. Philip, H. and M.N. Khan, J. Chem. Soc., Perkin Trans.I 809 (1975).

e. Ernest, W.C. and S. Dieter, J. Chem. Soc., Chem. Commun. 16:689 (1978).

76a. Haesner, A. and C.H. Heathcock, Tetrahedron Lett. 393 (1963).

b. Heathcock, C.H. and A. Haesner, Angew. Chem. 75:344 (1963).

c. Haesner, A. and C.H. Heathcock, Tetrahedron 20:1037 (1964).

d. Haesner, A., M.E. Lorder and C.H. Heathcock, J. Org. Chem. 32:540 (1967).

77a. Barton, D.H.R. and A.N. Starrett, J. Am. Chem. Soc. 87: 2444 (1965).

b. Tisue, G.T., S. Linke and W. Lwowski, ibid. 89:6303 (1967).

c. Lewis, F.D. and W.H. Saunders, Jr., ibid., 90:7033 (1968).

78a. Huisgen, R., Angew. Chem. 75:741 (1963).

b. L'abbe, G., Chem. Rev. 69:345 (1969).

79. Babu, M., Ph.D. Thesis, Aligarh Muslim University, Aligarh (1981).

80. Belinka, Jr., B.A. and A. Haesner, J. Org. Chem. 44: 4712 (1979).

81. Haesner, A. and F.W. Fowler, ibid. 33:2686 (1968).

82. Kabara, J.J., R. Vrable and M.S.F. Lie Ken Jie, Lipids 12:753 (1977).

83. Jain, P.C., Y. Khandelwal and O. Tripathi, J. Med. Chem. 21:68 (1978).

84. Bellamy, L.J., Advances in Infrared Group Frequencies, Chapman and Hall, London, p.123 (1975).

85. Stewart, J.E., J. Chem. Phys. 30:1259 (1959).

86. Bycroft, B.W., Nature 224:595 (1969).

87. Liesch, J.M. and K.L. Rinehart, Jr., J. Am. Chem. Soc. 99:1645 (1977).

88. Gebelein, C.G., G. Swift and D. Swern, J. Org. Chem. 32:3314 (1967).

89. Habourn, W.J. and W.L. Howard, J. Org. Chem. 27:1039 (1962).

90. Foglia, T.A., G. Maerker and G.R. Smith, JAOCS 47:384 (1970).

91. Colthup, N.B., L.H. Daly and S.E. Wiberley, Introduction to Infrared and Raman Spectroscopy, Academic Press, Inc. (NY), p.305 (1975).

92. Cromwell, N.H., R.P. Cahoy, W.E. Franklin and G.D. Mercer, J. Am. Chem. Soc. 79:922 (1957).

93. Maerker, G., E.T. Haeberer, E.T. Donahue and T.A. Foglia, J. Heterocyclic Chem. 7:563 (1970).

94. Artamonov, P.A., Zhur. Obschei. Khim. 22:1992 (1952).

Publications and Presentations

1. Quantitative Ring Cleavage of Long-Chain Epoxides by Chlorotrimethylsilane for Chlorohydrin Synthesis.
J. Oil Tech. Assn.(India) 13:135 (1981).

2. Epoxidation of Methyl 4-hydroxy-trans-2-hexadecenoate.
JAOCS 59:195 (1982).

3. Ochna arfopurpuria Seed Oil: A New Source of Palmitoleic Acid.
Chem. Ind.(London) 199 (1982).

4. Triglyceride Composition of Ochna squarrosa Seed Oil.
J. Oil Tech. Assn.(India) 14:127 (1982).

5. Cyclopropenoid Fatty Acids in Seed Oils of Urena reranda and Thespesia lampas.
JAOCS (In Press).

6. Chemical Screening of Seed Oils.
J. Oil Tech. Assn.(India) (In Press).

7. Synthesis of Tetrazoles from α,β-Unsaturated Carbonyl Fatty Acid.
JAOCS (Communicated).

8. Synthesis of Sulphur Heterocycles from α,β-Unsaturated Carbonyl Fatty Acid Ester.
ibid. (Communicated).

9. Iodoazide Addition to α,β-Unsaturated Fatty Alcohol, in 36th Annual Convention of Oil Technologists' Association of India (OTAI) held on Feb.14-15, 1981 at Hyderabad, India.

10. Chemistry of Long-Chain α,β-Unsaturated Acids: Epoxidation of Allylic Hydroxy Ester.
ibid.

11. Synthesis of N-Heterocyclic Derivatives from α,β-Unsaturated Carbonyl Fatty Acid, in 37th Annual Convention of OTAI held on Feb.13-14, 1982 at Kanpur, India.

12. Synthesis of Long-Chain Aziridines, in 2nd Annual Conference of Indian Council of Chemists' held on Nov.6-8, 1982 at Srinagar, India.

13. Synthesis of 3-Heterocyclic Derivatives from α,β-Unsaturated Oxo Fatty Acid.
ibid.